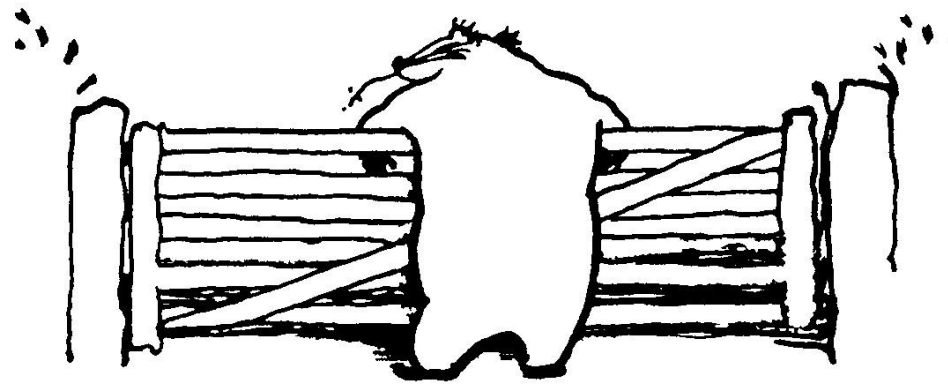


Recent advances in animal welfare science VIII



Scientific programme: Talks

UFAW Virtual Animal Welfare Conference

29th June -30th June 2021

#UFAW2021



Welcome to the Virtual UFAW Conference 2021

Welcome to UFAW's second online meeting. We are delighted that you can join us for what promises to be an exciting and engaging couple of days.

Switching to an online format has allowed us to reach a much larger and more global audience than we could at a face-to-face meeting, something we are incredibly excited about. We are being joined by attendees from at least 57 countries. The conference also features our largest ever number of presentations exploring a huge range of animal welfare issues. Please do ask questions of the speakers who will be available to answer them after their talks.

The scientific programme also includes 119 posters. Links to access these can be found at the bottom of each abstract in the poster programme booklet. Please visit the poster exhibition and leave comments for the authors to answer. You can also vote for the best poster - the winners and one lucky voter will win vouchers to spend on books from the [UFAW/Wiley-Blackwell Animal Welfare](#) book series.

We would like to thank all the participants and session chairs for their contributions to the meeting.

Please help us keep these meetings free and open to all.

A special thank you goes to all those who, when registering, made a donation – thank you – your support is already making a difference. Spreading the message about animal welfare science as far and wide as possible is one of UFAW's key objectives. We make our meetings free to ensure access for those who could not otherwise attend. However, running our meetings is costly and involves an enormous effort from UFAW's staff. As a charity, supported entirely by donations, we can only keep meetings free and run our other activities with your support. If you are in a position to do so, please consider making a donation to make sure that we can continue to make our meetings free to attend for all.

We would like to thank our publishing partner Wiley-Blackwell for their support. As part of your registration for this meeting Wiley-Blackwell are offering a discount of 20% on all the books in the [UFAW/Wiley-Blackwell animal welfare book series](#) (use the code VBT66 when you order from www.wiley.com).

We look forward to a thought-provoking couple of days and we hope that you all have an informative and enjoyable meeting.

Huw Golledge, Stephen Wickens, Birte Nielsen and Liz Carter
UFAW Organising Committee

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General Information

Organisers:

UFAW, the Universities Federation for Animal Welfare, is an UK based registered charity that works with the animal welfare science community worldwide to develop and promote improvements in the welfare of farm, companion, laboratory, captive wild animals and those with which we interact in the wild, through scientific and educational activity. To this end, UFAW:

- Promotes and supports developments in the science and technology that underpin advances in animal welfare, including the funding of research through its programme of [grants, awards and scholarships](#).
- Promotes education in animal care and welfare.
- Provides information, organises symposia, conferences and meetings, publishes books, videos, technical reports and the international quarterly scientific journal [Animal Welfare](#).
- Provides expert advice to governments and other organisations and helps to draft and amend laws and guidelines.
- Enlists the energies of animal keepers, scientists, veterinarians, lawyers and others who care about animals.

UFAW is an independent organisation, and throughout its history, its work has primarily been funded by [donations, subscriptions and legacies](#).

UFAW's philosophy: The importance of science to animal welfare:

Ensuring good welfare is about more than ensuring good health. Animal welfare is about the quality of animals' lives: their feelings. It is now widely agreed, although it is not yet possible to prove absolutely, that many species are sentient - they have the capacity to feel pain and distress, they can suffer and, conversely, be aware of pleasant feelings - and that this matters morally. But how do we assess, from the animal's point of view, what matters to them and how much?

"Science informs, motivates and facilitates advances in animal welfare by providing a strong evidence base for changing attitudes and practices, and by creating practical and effective solutions to welfare problems."

UFAW promotes and supports a scientific approach aimed at finding ways to gain insight into what matters to animals, assessing their welfare and improving the quality of their lives through practical developments in all aspects of their care.

Change for the better depends on knowledge, understanding and practical solutions. UFAW believes that good science can inform, motivate and facilitate that change - whether through developments in legislation, professional 'best practice' or the actions of other organisations and individuals.

In promoting and supporting this scientific approach to improving welfare, UFAW's work is wide-ranging and undertaken with many other organisations and individuals - enlisting and informing the energies of animal keepers, scientists, veterinarians, lawyers and others who care about animals.

For more details visit: www.ufaw.org.uk



SCIENTIFIC PROGRAMME:

Timetable and Speaker Abstracts

Recent advances in animal welfare science VIII

Virtual UFAW Animal Welfare Conference, 29th- 30th June 2021



Timetable

All timings are GMT+1 / UTC+1/British Summer Time

Day 1. 29 th June 11.00am – 18.20pm		
11.00 -11.10 Introduction to meeting		
	Huw Golledge UFAW, UK	Welcome and Introduction
11.10 – 13.00 Session 1 Chair: Huw Golledge (UFAW, UK)		
11.10	Cynthia Schuck-Paim and WJ Alonso (The Welfare Metrics Project, Spain)	The need for longitudinal studies in animal welfare science: research gaps in the study of individual suffering in laying hens
11.35	A Trevarthen, E Finnegan, K Bučková, A Resasco, E Paul, M Mendl and Carole Fureix (University of Bristol, UK)	Do antidepressant and/or environmental enrichment reduce the time mice spend inactive but awake in their home cage?
12.00	Winfried Otten, S Heimbürge, A Tuchscherer and E Kanitz (Leibniz Institute for Farm Animal Biology, Germany)	Hair cortisol levels in pigs and cattle: Effects of long-term stress and potential confounding factors
12.25	Short presentations: 1 Liza Moscovice, B Sobczak, C Gladbach, U Gimsa and E Kanitz (Leibniz Institute for Farm Animal Biology, Germany) 2 Rebecca King, SM Matheson, EM Baxter and SA Edwards (Newcastle University, UK) 3 David Massey, CL Witham and M Bateson (Newcastle University, UK) 4 Jakob Winter, A Stratmann, M Toscano and C Nicol (RVC, UK) 5 Ayoola Famakinwa and OA Adebisi (University of Ibadan, Nigeria) 6 Vladislava Milchevskaya, P Bugnon, E ten Buren, F Brand, A Tresch and T Buch (University of Cologne, Germany) Q&A	1. Salivary oxytocin co-varies with maternal investment in domestic pigs (<i>Sus scrofa domestica</i>): An assay validation with welfare implications 2. Sow behaviour and piglet weight gain after late cross-fostering in farrowing crates and pens 3. Effect of weaning (age) on alopecia in a UK breeding facility of rhesus macaques (<i>Macaca mulatta</i>) 4. Piling behaviour in British layer flocks: Description and characteristics 5. Size of enrichment material influences behaviour of growing pigs 6. Improved group size planning of breedings of gene-modified animals
13.00- 13.50 Break and poster viewing		
13.50 – 15.30 Session 2 Chair: Pol Llonch (UAB, Spain)		
13.50	Kate Lewis, SD McBride, MO Parker and L Proops (University of Portsmouth, UK)	Risk factors for stereotypic behaviour in captive ungulates
14.15	Daniel O'Hagan and TV Smulders (Newcastle University, UK)	Assessing transient affective state using intracranial recordings of brain oscillations in poultry
14.40	Andrea Polanco, DL Pearl, L Niel, B McCowan and GJ Mason (University of Guelph, Canada)	What can abnormal behaviour teach us about captive animals' quality of life? Validating stereotypic behaviour as a cumulative welfare indicator in laboratory rhesus monkeys (<i>Macaca mulatta</i>)
15.05	Aileen MacLellan, A Resasco, MA Ayala, L Kitchenham, AM Edwards, S Lam, S DeJardin and G Mason (University of Guelph, Canada)	Glass half full? Validation and application of a novel judgement bias task for mice
15.30 – 16.10 Break and poster viewing		
16.10 – 18.20 Session 3 Chair Claire Witham (MRC Harwell, UK)		
16.10	Grace Fuller and J Hamilton (Detroit Zoological Society, USA)	Exploring oxidative stress as a potential indicator of animal welfare
16.35	UFAW Award Presentations Joy Mench (University of California, Davis, USA) ***2021 winner - UFAW Medal for 'Outstanding Contribution to Animal Welfare Science'***	UFAW Medal for 'Outstanding Contribution to Animal Welfare Science' and 'UFAW Early Career Researcher of the Year'
17.25	Erin Ryan and D Fraser (University of British Columbia, Canada)	Designing animal welfare standards and audits - finding the right balance by optimizing the inclusion of resource-based and outcome-based measures
17.50	Short presentations: 1 Sophie Brajon, G Munhoz Morello, S Capas Penada, J Hultgren, C Gilbert and IAS Olsson (Universidade do Porto, Portugal) 2 Katherine Cronin, NK Wierzal, A Evans, M Ramont, M Leahy and JD Wark (Lincoln Park Zoo, Chicago, USA) 3 Vikki Neville, J Mouny, L Benato, K Hunter, M Mendl and ES Paul (University of Bristol, UK) 4 Sarah Huskisson, CR Doelling, SR Ross, and LM Hopper (Lincoln Park Zoo, Chicago, USA) 5 Claire Toinon, S Waiblinger and JL Rault (University of Veterinary Medicine, Vienna, Austria) Q&A	Non-target interactions and humaneness evaluation of captive bolt trap in rodent control 1. Newborn cannibalism in laboratory mouse and how it biases the estimation of pup mortality 2. Considering animal space use to evaluate and enhance welfare: examples from the zoo environment 3. Thinking outside the lab; can studies of pet rats inform laboratory rat welfare? 4. The impact of visitor effect on Japanese macaques' cognitive performance and welfare 5. Maternal deprivation affects goat kids stress coping behaviour

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Day 2. 30th June 08.20am – 16.00pm

08.20 – 09.45 Session 4 Chair: Mia Cobb (University of Melbourne, Australia)		
08.20	Introduction	
08.30	Isabella Clegg , HG Rödel, B Mercera, S van der Heul, T Schrijvers, P de Laender, R Gojceta, M Zimmiti, E Verhoeven, J Burger, PE Bunskoek and F Delfour (Animal Welfare Expertise, Sydney, Australia)	Dolphins' "Willingness to Participate" (WtP) in positive reinforcement training predicts early changes in health status
08.55	Dan O'Neill , C Pegram, P Crocker, DC Brodbelt, DB Church and RMA Packer (The Royal Veterinary College, UK)	Show me the brachy data! Are flat-faced dogs really unhealthier than other dogs?
09.20	Short presentations: 1 Sabir Hussain , A Hussain, J Ho, Jun Li, D George and OAE Sparagano (City University of Hong Kong, China; University of Veterinary and Animal Sciences, Lahore, Pakistan; Newcastle University, UK) 2 Edward Narayan , D Fox, G Sawyer and A Tilbrook (University of Queensland, Aus) 3 Aamer Khan , SEG Lea, P Chand, U Rai and N Baskaran (A.V.C. College (Autonomous), Mannampandal and Padmaja Naidu Himalayan Zoological Park, India; University of Exeter, UK) 4 Mona Giersberg , R Molenaar, IC de Jong, H van den Brand, B Kemp and TB Rodenburg (Utrecht University, Netherlands) 5 Evelien Graat , M Thys, L Warin, V Courboulay, M-C Meunier-Salaün, M Stomp, H Leruste, V Guesdon, A Collin, MF Giersberg, TB Rodenburg, F Tuytens and C Vanden (ILVO, Belgium) Q&A	1. Tick control in the bovine population of Pakistan: A step towards animal welfare 2. Tracking stress remotely via wool hormones and smart tags: shearing early in pregnancy reduces stress and improves productivity of Australian Merino sheep 3. Predictors of psychological stress and behavioural diversity among captive red panda in north-eastern India and their implications for global captive management 4. Effects of hatching system on broiler activity measured individually by an ultra-wideband tracking system 5. Development of tools for farmers to self-assess the welfare of their poultry and pigs in organic and outdoor systems

09.45- 10.30 Break and poster viewing

10.30 – 13.00 Session 5 Chair: Rebecca Nordquist (Utrecht University, The Netherlands)		
10.30	Jasmine Clarkson , JE Martin, J Sparrey, F Marchesi, MC Leach and DEF McKeegan (University of Glasgow, UK)	Behavioural, physiological and pathological assessment of decompression parameters for the potentially humane killing of anaesthetised laboratory rodents
10.55	Emmeline Howarth , C Kemp, HR Thatcher, ID Szott, D Farningham, CL Witham, A Holmes, S Semple and EJ Bethell (Liverpool John Moores University, University Centre Myerscough, University of Edinburgh, Medical Research Council Harwell, Newcastle University and University of Roehampton, UK; Unitec Institute of Technology, NZ)	Developing and validating attention bias tools for assessing trait and state affect in animals: A worked example with <i>Macaca mulatta</i>
11.20	Tori McEvoy , U Baqueiro - Espinosa, A Crump and G Arnott (Queen's University Belfast, UK)	Benefits of a socialisation protocol on commercially bred dogs
11.45	Lina Weirup , C Schulz and H Seibel (Gesellschaft für Marine Aquakultur, Germany)	Fish welfare evaluation index based on the prevalence and severity of external morphological damage
12.10	Deborah Nadal (University of Glasgow)	When "One Health, One Welfare" encounters rabies in the streets of India
12.35	Short presentations: 1 Janire Castellano Bueno , A Paraskevopoulou, L Sermin-Reed, S Groves, N Kindred, M Jay, A Czeszyk, J Panafieu, J Safourcade, S Carella, C Miller, M Bateson and C Poirier (Newcastle University, UK) 2 Rhyanne Heppenstall , S Minano, A Fisk, M de Vos, R Austin, M Bonsall and CE Bergmann (University of Oxford, UK) 3 Charles Carslake , JA Vázquez-Diosdado and J Kaler (University of Nottingham, UK) 4 Adam Powell (University of Stirling, UK) 5 Tristram Wyatt (University of Oxford, UK) Q&A	1. Acute effect of fluid control on the welfare of laboratory rhesus macaques 2. Accelerometers as tools to monitor behaviour: a potential refinement for the welfare assessment of rhesus macaques used in neuroscience research 3. Machine learning algorithms to classify and quantify multiple behaviours in dairy calves using a sensor: Moving beyond classification in precision livestock 4. Rubber net mesh reduces scale loss in farmed Atlantic salmon, <i>Salmo salar</i> 5. Reproducibility and open science: Learning from psychology

13.00- 14.00 Break and poster viewing

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14.00 – 16.00 Session 6 Chair: Birte Nielsen (UFAW, UK)		
14.00	Jamie Ahloy Dallaire (Université Laval, Canada) ***2021 UFAW Early Career Researcher of the Year' winner***	Identifying and raising adaptable animals
14.25	Carly O'Malley , LM Wilkinson, ER Moreau and PV Turner (Charles River Laboratories, USA)	Driving organizational change in welfare practices: A stakeholder focus group approach
14.50	Short presentations: 1 Fiona Dale , CC Burn, J Murray and R Casey (RC, UK) 2 Sara Owczarczak-Garstecka , RA Casey, RH Kinsman, TG Knowles, S Tasker, REP Da Costa and JK Murray (Dog Trust, UK) 3 Martine Van den Broeck and P Cornillie (Ghent University, Netherlands) 4 Guillermina Hernandez-Cruz , RG Ferreira, N Rooney, TSF Costa, DR Rego and M Mendl (University of Bristol, UK) Q&A	1. Changes in separation related behaviour in puppies aged from 12 weeks to 6 months of age: description of a 'Generation Pup' cohort 2. Owner-reported post-neutering care and wound problems for dogs at ages 7 and 15 months 3. Discrepancy between legal and actual age of puppies in cross-border trade 4. Behavioural diversity of capuchin monkeys (<i>Sapajus libidinosus</i>) in rehabilitation
15.10	Jen-Yun Chou (University of Pennsylvania, USA) ***2021 UFAW Early Career Researcher of the Year' winner***	If you judge a pig by the tail" - how a solution-driven approach can open up new possibilities to animal behaviour and welfare research
15.35	Raúl Guevara , JJ Pastor, X Manteca, G Tedo and P Llonch (Universitat Autònoma de Barcelona, Spain)	Systematic review of animal-based indicators to measure thermal, social, and immune-related stress in pigs
16.00 End		



THE NEED FOR LONGITUDINAL STUDIES IN ANIMAL WELFARE SCIENCE: RESEARCH GAPS IN THE STUDY OF INDIVIDUAL SUFFERING IN LAYING HENS

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Pain and suffering are concepts that inherently concern individuals. It is at the individual level where cognitive and sensorial mechanisms are integrated, making the emergence of states such as joy, pain and fear possible. However, despite substantial progress over the last decades in the understanding of the welfare challenges affecting animals, their risk factors, prevalence and heritability, few are the welfare harms for which information about their effects at the individual level (e.g., clinical evolution, duration of symptoms, healing times, rates of recurrence) is available, with even less studies exploring the possible intensity and duration of the pain they impose. We have recently developed an analytical framework to quantify the loss of welfare experienced by animals based on estimates of the intensity and duration of the pain endured as a result of each of different sources of physical and psychological pain that individuals are exposed to and applied it to quantify the loss of welfare experienced by commercial laying hens in different housing systems. The need to quantify the intensity and duration of the pain emerging from multiple injuries, diseases, and deprivations has highlighted different research areas where knowledge is still lacking, which will be presented and discussed. For example, despite the extensive literature on injurious pecking and cannibalism, little information is available on the time course of fatal cannibalistic attacks. Similarly, little is known about the clinical evolution of septicemia in chickens (an extremely painful condition), even though it is one of the most frequent causes of carcass condemnation in the poultry industry. Longitudinal studies targeting individual birds in commercial conditions are therefore very much needed. They can help understand (1) the extent to which each welfare harm disrupts the behavior of individuals affected, helping establish the degree to which conditions are perceived as aversive; (2) the recurrence of harms (e.g., how many injuries or disease episodes individuals typically endure, and how recurrence varies across individuals); (3) the temporal evolution of each harm, including fatal conditions; (4) rates of spontaneous healing; (5) the extent to which behavioral opportunities are realized in different conditions (e.g. housing systems), and how it changes over time; (6) patterns of comorbidity and (7) synergistic effects. Importantly, longitudinal studies could help identify those individuals disproportionately affected by the pressures and hazards imposed by different living conditions. The availability of methods to tag and video-record animals should greatly facilitate efforts in this direction.

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DO ANTIDEPRESSANT AND/OR ENVIRONMENTAL ENRICHMENT REDUCE THE TIME MICE SPEND INACTIVE BUT AWAKE IN THEIR HOME CAGE?

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We previously identified in laboratory mice an inactive state [being awake with eyes open motionless within the home cage, inactive but awake 'IBA'], triggered by barren cages and associated with a key feature in animal models of human depression: cessation of swimming in forced-swim tests. We tested further the hypothesis that greater time spent displaying IBA indicates a depression-like state in mice by testing whether IBA is alleviated by giving antidepressants that are clinically efficient in humans, by providing less stressful life conditions, and whether combining these therapeutic approaches magnifies their effects. Seventy-two C57BL/6J and 72 DBA/2J female mice were pseudo-randomly housed in mixed strain-pairs in small non-enriched (NE) or in large environmentally enriched (EE) cages from 3-4 weeks of age. After 34 days, half of the mice housed in NE cages were either relocated to EE cages or left in NE cages. For each of these conditions, half of the mice were trained to drink from a syringe either a placebo (n=24 mice) or the antidepressant Venlafaxine (10mg/kg, n=24). The 48 mice housed in EE cages were all relocated to NE cages and allocated to either a placebo treatment (n=24), or Venlafaxine (n=24). In-cage IBA data were collected by trained observers using a mix of live scan- and focal-sampling in two daily 90-minute time blocks repeated over 2-4 days per week, over 3 consecutive weeks before and after environmental adjustment. Experimenters were blind to the pharmacological treatment, and behavioural observers were blind to both pharmacological and environmental adjustment treatments. Data were analysed using generalised linear mixed models. As expected, NE cages triggered IBA more than EE cages (LRTChi²₆ = 2.768, p<0.0001). For mice originally housed in NE cages, the proportion of visible scans displaying IBA increased over time only in mice from the NE-NE-Placebo control group, while IBA decreased following pharmacological, environmental enrichment, and both treatments (LRTChi²₉ = 0.850, p=0.003). Importantly, Venlafaxine and environmental enrichment appear equally as effective at reducing IBA, and combining these approaches did not magnify reduction in IBA (post-hoc comparisons, p=0.2050 to 0.5816). Moving mice from EE to NE cages induced a significant increase in IBA (LRTChi²₅ = 0.932, p<0.001), with no significant difference in the rise shown by the placebo and drug groups (LRTChi²₃ = 0.024, p=0.864). Venlafaxine therefore did not overcome the increase in IBA resulting from enrichment loss. Theoretical and practical implications for mouse welfare, and further research directions, will be discussed.

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HAIR CORTISOL LEVELS IN PIGS AND CATTLE: EFFECTS OF LONG-TERM STRESS AND POTENTIAL CONFOUNDING FACTORS

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The detection and avoidance of distress is one demand for an improved welfare of animals. Recently, analysis of hair cortisol concentration (HCC) has gained increasing attention as a promising and minimally invasive method for the appraisal of long-term stress. However, before HCC can be applicable, the validity of this indicator has to be elucidated and potential confounding factors have to be determined. Therefore, the first aim of our study was to validate HCC in pigs and cattle by using repeated ACTH administrations to simulate stress-induced cortisol release. For this purpose, 34 cattle and 38 gilts were treated either with i.m. injections of ACTH or saline every second day over four weeks. At the end of the treatment (week 4) and after 8 and 12 weeks, samples of natural and regrown hair were taken and hair segments were collected after 12 weeks. Additionally, an *in vitro* experiment was performed to investigate the potential confounding impact of contamination with urine, saliva and faeces on cortisol levels in hair samples of 12 pigs and 12 cattle. Samples were contaminated over 20 days for two hours daily. All hair samples were washed, ground, extracted and analysed for HCC by an enzyme immunoassay. Statistical analysis was conducted with the MIXED procedure of SAS/STAT software using ANOVA and pairwise comparisons by Tukey Kramer tests. The results from our ACTH experiment show significantly increased cortisol levels in all hair types of ACTH-treated cattle compared with controls ($p < 0.001$). In pigs, we observed an increase of HCC in both treatments, probably due to cross-contamination by urine and saliva. The *in vitro* contamination of hair with urine caused a considerable increase of HCCs in both species ($p < 0.05$), whereas saliva and faeces enhanced HCC only in hair of cattle ($p < 0.01$). Our findings provide evidence that hair cortisol reflects preceding periods with increased cortisol release and may thus be a promising indicator of stress, especially in cattle. However, in pigs housed in commercial husbandry conditions, contamination of hair may affect HCC and thus compromise its validity as an indicator of stress.

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SALIVARY OXYTOCIN CO-VARIES WITH MATERNAL INVESTMENT IN DOMESTIC PIGS (*SUS SCROFA DOMESTICUS*): AN ASSAY VALIDATION WITH WELFARE IMPLICATIONS

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Assessments of emotional states in animals can be achieved by measuring coordinated changes in behavior, physiology and/or cognition in response to specific events. Oxytocin (OXT) is an evolutionarily-conserved hormone that mediates species-typical social behaviors related to reproduction, maternal care and affiliation more generally, making it a promising candidate for assessing positive emotional states. Our goals were to validate a non-invasive measure of salivary OXT in domestic pigs, and to assess whether salivary OXT co-varies with maternal behavior. Our study subjects were $n = 22$ sows housed at our Experimental Pig Facility in conventional farrowing pens, including a guard rail that partially restricts sow movement for ten days following birth. We observed eleven sows regularly during their lactational period and recorded the hourly proportion of time that piglets had contact with teats (nursing contact), or had any physical contact with the sow (social contact). Following each behavioral observation, sows voluntarily chewed on swabs (SalivaBio®, Salimetrics) to provide saliva samples ($n = 119$). We also performed a biological validation by measuring changes in salivary OXT during parturition in an additional eleven sows. Saliva samples were extracted and measured using an Enzyme Immunoassay (Cayman Chemical). Our validations of parallelism, repeatability and recovery confirmed that the assay is suitable for measuring OXT in pig saliva. In addition, our biological validation confirmed that OXT increases in saliva of sows coinciding with parturition (Wilcoxon, $p = 0.01$). Sows' salivary OXT concentrations were predicted by their extent of nursing contact in the previous hour (GLMM, $p = 0.03$), but not by their overall social contact with piglets. We conclude that fluctuations in salivary OXT in sows reflect variation in maternal investment. Follow-up studies will investigate the relationship between sow OXT and maternal behavior in alternative farrowing systems where sows have more control over their interactions with piglets.

This research was funded by UFAW Grant Award 44-19/20.

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SOW BEHAVIOUR AND PIGLET WEIGHT GAIN AFTER LATE CROSS-FOSTERING IN FARROWING CRATES AND PENS

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Late cross-fostering of piglets, after the first 24 hours post-partum, is routinely practiced on many commercial farms. Whilst much research has looked at the immediate consequences of late cross-fostering on piglet weight gain, these studies were all conducted in farrowing crates, and with little focus on the longer-term impacts on piglet weight gain or the role of the sow's behaviour on piglet outcomes. With sustained interest in free-farrowing, any detrimental consequences of late cross-fostering due to the sow's behaviour may be worsened in such systems due to the increased behavioural freedom afforded to the sow. The current study used a 2x2 factorial design to compare sow postures, piglet-directed and nursing behaviour (n=48) after litter reunion, plus piglet average daily gain (ADG) and total weight gain (TWG) throughout the lactation (n=539) and weaner periods (n=108), after actual or sham late cross-fostering of four piglets per litter, performed six days post-partum, amongst litters housed in farrowing crates or free-farrowing (PigSAFE) pens.

Post-reunion, piglet-directed sniffing behaviour was higher amongst penned than crated sows ($P < 0.001$) and amongst foster than control sows ($P < 0.01$). Post-reunion, penned sows spent more time standing ($P < 0.01$) and less time sitting ($P < 0.01$) than crated sows, whilst foster sows spent more time standing ($P < 0.01$) and less time lying down ($P = 0.01$) than control sows. All sows showed a similar latency to the first nursing bout ($P > 0.10$), however the latency to the first successful nursing bout was affected by a housing and treatment interaction ($P < 0.001$), being shortest and longest for crated control and crated foster sows, respectively. During days 6-8 post-partum, piglet ADG was lower amongst cross-fostered piglets ($P < 0.001$) and their littermates ($P < 0.05$) in both housing systems. Lactation TWG (days 6-26) was higher amongst pen-housed control piglets than both pen-housed ($P < 0.01$) and crate-housed ($P < 0.05$) cross-fostered piglets, whilst crate-housed control piglets were intermediate. Post-weaning TWG (days 26-60) was higher amongst cross-fostered than control piglets ($P < 0.01$), irrespective of the rearing system.

In conclusion, late cross-fostering had an immediate effect on sow behaviour and piglet weight gain in both housing systems, with a more prolonged negative effect on pre-weaning piglet weight gain in the farrowing pens. However, after weaning, the total weight gain of cross-fostered piglets exceeded that of controls from both rearing systems.

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**EFFECT OF WEANING (AGE) ON ALOPECIA IN A UK BREEDING FACILITY OF RHESUS
MACAQUES (*MACACA MULATTA*)**

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Alopecia occurs in both humans and nonhuman primates (herein, primates). In primates, alopecia has been shown to be associated with a number of variables including sex, season, pregnancy, hair pulling etc. Furthermore, severe alopecia (>30% hair loss) has been identified as a potential biomarker of psychological stress. The age at which captive rhesus macaques (*Macaca mulatta*) are permanently separated from their mother (herein, weaned) is a major stressor in the life history of captive macaques and has been identified as a candidate for refinement. Here, we present our findings on the association between weaning (age) and alopecia in a UK breeding colony of indoor group-housed rhesus macaques. We examined historical colony records of annual health screens (HS) from 2010 – 2019 that include alopecia scores as a measure. We took a sample of females that had observations for two consecutive HS (n = 90). Some females remained with their mother for both HS (n = 47), the remainder were weaned at least 60 days prior to the second HS (n = 43). Age ranges of all females during the first HS were 1.53 – 3.91yrs (mean±SE = 2.11±0.05) and alopecia scores did not differ between groups at the first HS. At the second HS, unweaned female ages ranged from 2.31 – 4.8yrs (mean±SE = 3.1±0.09); weaned female ages ranged from 2.43 – 3.97yrs (mean±SE = 2.98±0.06); and weaning ages ranged from 1.65 – 3.43yrs (mean±SE = 2.3±0.06). We modelled the impact of weaning and weaning age on alopecia scores. To model the effect of weaning age on the magnitude of hair loss, we created an alopecia difference score (first HS – second HS). Season and age at HS were included in all models as covariates. We found that weaned females at the second HS had more alopecia (estimate±SE = 0.61±0.22, z = 2.78, p = 0.005), a later weaning age was associated with less alopecia (estimate±SE = -0.37±0.18, z = -2.06, p = 0.04), and a later weaning age was associated with a positive difference score which indicated hair gain between HS, although this effect was not statistically significant (estimate±SE = 0.63±0.31, t = 2.02, p = 0.0503). These results provide evidence that a later weaning age could mitigate the negative effects associated with weaning in indoor group-housed female rhesus macaques.

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PILING BEHAVIOUR IN BRITISH LAYER FLOCKS: DESCRIPTION AND CHARACTERISTICS

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Creeping smothering, the unpredictable suffocation of laying hens, is a well-known phenomenon experienced by many British loosed-house egg-producers. The underlying mechanism is piling behaviour (PB) where several hens mass together in the litter area. Despite animal welfare concerns, characteristics (i.e., frequencies, durations, and number of piling birds) and events preceding PB on British layer flocks have not yet been investigated. Studies from Swiss flocks show that PB can occur frequently and be elicited by various events. However, extrapolation of these findings to British flocks is difficult due to housing differences (e.g., flock sizes, breeds) likely influencing PB. The objectives of this study were to describe PB characteristics and preceding events on British layer flocks. Following an exploratory approach, the behaviour of 20 loosed-house flocks (12 free-range, 8 organic, size (mean \pm SD) 7600 \pm 5800 hens) belonging to two egg-producer organisations and known to repeatedly experience smothering, was recorded. The flocks were visited once between January and August 2020 at the beginning of lay (20.5 \pm 2.6 w) or a later point (33.0 \pm 6.9 w). In each shed, two corners of the litter area and (only for free-range flocks) three locations in the centre of the shed were video recorded from 08:30 - 16:00 h. Piling characteristics and preceding events were assessed for one day per flock. PB was defined as more than two mostly immobile hens standing in closest possible proximity with heads facing mostly in the same direction. Videos were analysed by one-minute scan sampling, wherefore only piles >1 min were taken into consideration. In total, 97 PB events were identified. Piles occurred in nearly all free-range (11 flocks, 77 piles) and half of organic (4 flocks, 20 piles) systems, in the centre (45.3%) of the litter area, in shed corners (31.9%) and along walls (22.6%). On average, piles lasted 27.9 \pm 74.7 min and involved 58 \pm 90 animals. Four events preceded PB, mostly hens joining various other hens' behaviours (e.g., sitting, standing, 59.8%) and high animal densities in the litter area (22.7%). Piles started more frequently before noon (10:30 - 12:30, 33 piles) and in the early afternoon (12:30 - 14:30, 29 piles) compared to the morning (08:30 - 10:30, 24 piles) and afternoon (14:30 - 16:00, 12 piles). At the beginning of lay, in forty percent of flocks (6 flocks, 35 piles), piles lasted longer (45 \pm 117.8 min) and involved more animals (96 \pm 131 animals) compared to later in lay (9 flocks, 62 piles, 18 \pm 27.5 min, 36 \pm 42 animals). Overall, PB was mostly preceded by hens joining other hens' behaviour and high animal densities in the litter area and showed daytime and flock age related variations.

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SIZE OF ENRICHMENT MATERIAL INFLUENCES BEHAVIOUR OF GROWING PIGS

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Behavioural studies involving giving animals opportunities and choices to express preferences have been found to provide information about their welfare. Studies on environmental enrichment in growing pigs in Nigeria are scanty. Therefore, this experiment was conducted to study the effect of enrichment sizes on behavioural pattern of growing pigs. A total of sixty crossbreeds (Largewhite X Landrace) grower pigs of 19-20kg were randomly distributed into five treatments in triplicates with four pigs per replicate arranged in completely randomized design for six weeks. Three different sizes of soft woods were used as enrichment: 40.0 x 4.0 x 4.5cm (small wood: SW), 55.0 x 5.0 x 4.5cm (medium wood: MW) and 70.0 x 6.0 x 4.5cm (large wood: LW) suspended on each side by 6mm blue rope to the shoulder height of the experimental animals for easy accessibility. The treatments are T1: control (without enrichment), T2: three pieces of SW, T3: three pieces of MW, T4: three pieces of LW, T5: Composite-Wood CW (1SW+1MW +1LW). Behavioural observations were monitored with CCTV and recordings were made for 6 hours/day (09:00-12:00 and 15:00 - 18:00) in 3 days/week. Parameters measured were Pen-Mate Manipulation (PMM): nosing, biting, chasing and rubbing; Pen Component Manipulation (PCM): nose or mouth in contact with pen fittings, wall and floor; and Enrichment Use (EU): rooting substrates, nosing substrates and chewing substrates; these were analysed using repeated measures while effect of time was analysed using t-test. Performance indices measured were Weight Gain-WG (kg), Final Weight-FW (kg) while the Feed Conversion Ratio (FCR) was calculated, these were analysed using ANOVA. The PMM (12.63%) and PCM (55.96%) in control were significantly ($p<0.05$) higher across the treatments, while EU (33.29%) was highest for pigs in CW. The PMM (8.78%) and PCM (42.90%) were significantly ($p<0.05$) higher at 09:00-12:00 hours than at 15:00-18:00 hours (7.71% and 41.97%, respectively). The WG of 9.04 (control) and 9.18 (SW) were significantly lower than 10.49 (LW), 10.53 (MW) and 10.57 (CW) this followed the same trend as the FW. Pigs on SW (3.27) recorded highest FCR which was significantly similar to control (3.14). The findings of the study revealed that, size of enrichment had significant effects on the behaviour of pigs, with pigs in MW and CW eliciting appropriate stimulus which could serve as a means of preventing pigs from performing adverse behaviours towards pen-mate and pen component, consequently interacting more with the enrichment objects provided.

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IMPROVED GROUP SIZE PLANNING OF BREEDINGS OF GENE-MODIFIED ANIMALS

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Biomedical research relies heavily on the classical vertebrate mouse model, even more since genome editing allows the rapid generation of mutant strains and complex genotypes. In this light, animal welfare and efficient experiment planning become critical. To obtain the desired cohort sizes for the wanted genotypes, one needs to account for the effective genotype frequencies in the offspring, the fertility rate of the respective mouse strain and the variation in litter size. Naïve expectation values often do not conform to the actual breeding outcome in practice, with small breeding setups being affected the most. Therefore, breeding processes have been facing delays, inflation of animal use and cohorts with strongly varying ages.

Here we optimise the calculation of the number of the breedings needed for having certain success guarantees. Primarily for small breeding setups, this approach is advantageous compared to the conventional techniques, as it allows researchers to reduce animal use without compromising the confidence to obtain the desired breeding outcome. Although less critical, we also observe an improvement in animal use for large breeding setups. Additionally, we verify our method by accessing the empirical distributions of the litters for various mouse strains.

For practical application, we developed an R package and Shiny Web application: These tools should facilitate the accurate calculation of the breeding outcomes, thereby optimising animal use and making breeding experiments more time- and cost-efficient.

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RISK FACTORS FOR STEREOTYPIC BEHAVIOUR IN CAPTIVE UNGULATES

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Stereotypic behaviours are widely reported across a variety of captive species, with significant implications for the management of these animals including increased injuries and poor physical health, reduced production or sporting performance, decreased financial worth, and negative public attention. Stereotypic individuals often demonstrate reduced behavioural flexibility, which may hamper conservation and reintroduction efforts. Captive ungulates are kept as production animals, in zoos, and for transport, sport and leisure, and can display a range of stereotypic behaviours, including tongue rolling, pacing and crib-biting. Although stereotypies in livestock and horses are well studied, data regarding stereotypies for the majority of exotic ungulate species are sparse, and there are vast inter-species differences in their type, prevalence and frequency.

We aimed to identify which aspects of ungulates' natural behavioural are the strongest predictors of stereotypy propensity by conducting two systematic literature reviews to collect stereotypy prevalence and performance data, and information regarding species' wild behavioural ecology. BIAZA*-accredited institutions were also contacted to gain unpublished data regarding stereotypies. High quality stereotypy data (from 222 sources) were available for 41 species. We hypothesised that risk factors for stereotypy development would fall into three key categories: (1) those relating to foraging, eating and processing feed substrates; (2) disparities between a species' natural social organisation and their captive conditions; and (3) movement and natural range size. We further hypothesised that the nature of the stereotypy observed (oral versus locomotor) may reflect the type of risk factor identified.

We ran a series of Bayesian models in Stan computational framework, accessed with R package *brms*, controlling for phylogenetic relatedness of species. As hypothesised, some measures of wild movement, foraging and social behaviour influenced stereotypy prevalence in captive ungulates, specifically, the proportion of the day spent active (95% CI 0.44, 2.96]), feeding strategy (95% CI [0.54, 2.04]), and engaging in a promiscuous mating strategy (95% CI [16.52, 125.39]). We also found, in agreement with previous studies, that feeding in meals as opposed to ad libitum (95% CI [27.21, 63.24]), and feeding concentrates (95% CI [24.46, 100.94]) in captivity are risk factors for stereotypy. Variables that were not predictive of stereotypy included home range size, daily travel distance, and proportion of the day spent eating. This exploratory work paves the way towards a deeper understanding of how divergence from free ranging ecologies result in discrete stereotypies in captive animals, providing the potential for targeted enrichment to improve the welfare of ungulate species.

* British and Irish Association of Zoos and Aquariums

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ASSESSING TRANSIENT AFFECTIVE STATE USING INTRACRANIAL RECORDINGS OF BRAIN OSCILLATIONS IN POULTRY

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Broiler chickens are exposed to many short term processes prior to slaughter, e.g. capture, loading into crates, transportation, and lairage. Many of these processes are likely to induce stress. The impact of these processes on broiler welfare could be substantial, but this area is poorly studied. One potential method of assessing welfare is to establish and implement electrophysiological markers of affective state.

Broilers were implanted with intracranial electrodes targeting key brain regions (Nidopallium Caudolaterale (NCL), Nucleus Taeniae (TnA) and the hippocampal formation (HF)) suspected to be involved in processing affective state, and subcutaneous heart rate loggers. Local field potentials were recorded from these brain areas in awake and behaving chickens and stored on a wireless logger. During a seven day experimental period, focal chickens were exposed to three negative experiences and one positive experience twice per day. These experiences were being held upside-down; being separated from their flock mates; having their comb pinched; and being fed mealworms.

In all three brain areas, theta and alpha power decreased from baseline levels during all conditions, however, the most marked decrease often occurred when the experimenter entered/left the room or approached the pen. Heart rate did not deviate from baseline levels during isolation but increased during the other conditions. Overall changes in theta and alpha power relative to baseline likely reflected arousal, rather than the valence of the conditions experienced. Alpha band coherence between the right and left TnA, right and left HF and left TnA and HF was significantly higher than at baseline when mealworms were presented. This increase in coherence was significantly different from the changes in coherence during the negatively-valenced conditions (inversion, isolation and pinch). Changes in coherence between these brain areas may thus dissociate between the experience of positive and negative affective states in broiler chickens

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WHAT CAN ABNORMAL BEHAVIOUR TEACH US ABOUT CAPTIVE ANIMALS' QUALITY OF LIFE? VALIDATING STEREOTYPIC BEHAVIOUR AS A CUMULATIVE WELFARE INDICATOR IN LABORATORY RHESUS MONKEYS (*MACACA MULATTA*)

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Over their lives, laboratory monkeys experience many welfare-relevant events that determine their 'cumulative welfare': whether their lives are worth living in terms of net positive (rewarding) and negative (aversive) experiences. Good management would retire animals before cumulative welfare becomes severely low, but how do we measure this objectively? Using cage-side behavioural assessments, we tested the hypothesis that motor stereotypic behaviour (MSB) indicates cumulative welfare in 919 rhesus monkeys from the California National Primate Research Center. This hypothesis predicts that MSB is better explained by the sum of life events than just demographic characteristics. It also predicts that MSB will be reduced by events pre-determined to be good for welfare (e.g., outdoor-mother rearing and an older age when removed from the dam), and increased by events pre-established to be bad for welfare (e.g., nursery rearing and single housing). Mixed logistic models revealed novel results regarding the effects of demographic variables. MSB risk increased with age among most monkeys, unless they were field-cage reared, in which case MSB risk decreased with age. The risk of MSB was also higher in female monkeys whose dams showed MSB. Further, monkeys whose sires did not perform MSB displayed decreased MSB risk with past time spent single-housed, suggesting that a non-stereotypic sire protects offspring from the SB-inducing effects of single housing. Additionally, consistent with the hypothesis, model fit (i.e., Akaike information criteria) improved when considering the sum of life events compared to a model only containing sex and age. Looking at life events, we found that maternal separation between 12-24 months of age and past housing near the door (both bad for welfare) significantly increased MSB risk. However, some events detrimental to welfare unexpectedly had no significant effect on MSB risk (e.g., time spent hospitalized and the number of location moves), while other negative events significantly reduced MSB risk (e.g., past health events and single housing). When analyzing interactions, some negative life events (e.g., nursery rearing, project count, and current single housing), either increased or decreased the risk of MSB, depending on the level of another life event or demographic characteristic. In conclusion, the hypothesis was rejected because not all life events predicted MSB in the expected direction, suggesting that MSB would make a poor diagnostic of cumulative welfare in other rhesus populations. Why some negative events reduced MSB risk requires more research into inactive responses to chronic stress.

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GLASS HALF FULL? VALIDATION AND APPLICATION OF A NOVEL JUDGEMENT BIAS TASK FOR MICE

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In humans, affective states can influence responses to ambiguous information: a phenomenon known as judgment bias (JB). Such JBs can reflect both the valence and intensity of affective states and thus have great potential as a tool for animal welfare assessment. In animals, JBs can be assessed by training individuals to perform an operant response to one cue to receive a reward, and another cue to avoid punishment, before assessing responses to intermediate, ambiguous cues. If a task has construct validity, then similar to humans, animals experiencing positive affective states will show positive biases, responding 'optimistically' to ambiguous stimuli as if expecting reward. Conversely, animals in negative affective states will demonstrate negative biases, behaving 'pessimistically' as if expecting punishment (or no reward). Evidence of construct validation is essential for any new animal JB task. However, for laboratory mice (*Mus musculus*), the most widely used vertebrate in research, successful validation of a JB task has proved elusive. Here, we aimed to validate a novel JB task by assessing its sensitivity to the effects of environmentally enriched and conventional barren laboratory cages- a system that reliably induces changes in mouse affect. Using a Go/Go design, C57BL/6 and Balb/c females were trained to discriminate between mint and vanilla odour cues and dig for high- or low-value food rewards. There were differences observed between the discriminative stimuli used, mice assigned mint as their positive cue interpreting ambiguous odour mixtures as positive. However, for mice with vanilla as their positive discriminative stimulus, the predicted housing effects were observed: mice housed in enriched conditions demonstrating relative optimism through shorter latencies to dig, as if expecting high-value rewards ($p=0.014$, *Cohen's d*=1.148). Having achieved construct validation, we then used a shortened protocol to test for 'pessimism' in mice implanted with subcutaneous lung adenocarcinomas. Tumour-bearing males (albeit not females) interpreted ambiguous cues more pessimistically than healthy controls ($p=0.005$, *Cohen's d*=1.425). To our knowledge, this is the first validation of a JB task for mice, and the first potential evidence of pessimism in tumour-bearing animals. Refinements to improve its sensitivity are still needed, but this promising new JB task has great potential for assessing mouse welfare and addressing fundamental questions about affective state.

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**UFAW MEDAL FOR 'OUTSTANDING CONTRIBUTION TO ANIMAL WELFARE SCIENCE'
AND UFAW EARLY CAREER RESEARCHER OF THE YEAR**

The **UFAW Medal for Outstanding Contributions to Animal Welfare Science** is a prize that recognises the exceptional achievements of an individual scientist who has made fundamental contributions to the advancement of animal welfare over a number of years. The award is open to individuals whose research, teaching, service and advocacy has had international impact and significantly benefited the welfare of animals. Previous winners have been:

- [2020 Professor Daniel Weary \(University of British Columbia, Canada\)](#)
- [2019 Professor Paul Hemsworth \(University of Melbourne, Australia\)](#)
- [2018 Professor Paul Flecknell \(Newcastle University, UK\)](#)
- [2017 Professor Sandra Edwards \(Newcastle University, UK\) and Professor Jeff Rushen \(University of British Columbia, Canada\)](#)
- [2016 Professor Donald Broom \(University of Cambridge, UK\) and Professor Christopher Wathes \(The Royal Veterinary College, UK\)](#)
- [2015 Professor David Mellor \(Massey University, New Zealand\) and Professor Georgia Mason \(University of Guelph, Canada\)](#)
- [2014 Professor Mike Mendl \(University of Bristol, UK\) and Professor David Fraser \(University of British Columbia, Canada\)](#)
- [2013 Professor John Webster \(University of Bristol, UK\) and Professor Peter Sandøe \(University of Copenhagen, Denmark\)](#)
- [2012 Professor Christine Nicol \(University of Bristol, UK\) and Professor Marian Stamp Dawkins \(University of Oxford, UK\)](#)
- [2011 Professor Ian Duncan \(University of Guelph, Canada\)](#)

The **UFAW Early Career Researcher of the Year Award** is a prize that recognises the achievements of young scientists who have made significant contributions to improving the welfare of animals. The award is open to students who are currently studying for a doctoral degree and to individuals who are within six years of the end of their PhD work.

Previous winners have been:

- [2020 Dr Irene Camerlink \(Polish Academy of Sciences, Poland\)](#)
- [2019 Dr Marisa Erasmus \(Michigan State University, USA\)](#)
- [2018 Dr Rebecca Meagher \(University of Reading, UK\)](#)
- [2017 Dr Pol Llonch \(Universitat Autònoma de Barcelona, Spain\)](#)
- [2016 Dr Rowena Packer \(The Royal Veterinary College, UK\)](#)
- [2015 Dr Jasmeet Kaler \(University of Nottingham, UK\)](#)
- [2014 Dr Lisbet Pluym \(Ghent University, Belgium\)](#)
- [2013 Dr Nuno Franco \(Institute of Molecular and Cell Biology, Porto, Portugal\)](#)
- [2012 Dr Charlotte Burn \(The Royal Veterinary College, UK\)](#)
- [2011 Dr Lucy Asher \(University of Nottingham, UK\), Dr Emma Baxter \(Scottish Agricultural College, UK\) and Dr Lisa Collins \(Queen's University Belfast, UK\)](#)

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DESIGNING ANIMAL WELFARE STANDARDS AND AUDITS - FINDING THE RIGHT BALANCE BY OPTIMIZING THE INCLUSION OF RESOURCE-BASED AND OUTCOME-BASED MEASURES

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Animal welfare standards and audits have often had a significant focus on specifying the quality and quantity of resources to be provided to the animals. However, making progress in improving welfare has sometimes proven to be challenging even when empirically validated resource-based standards are applied, due to the multi-factorial nature of some of the more pressing animal welfare problems (e.g., lameness, abnormal behavior). Attention has therefore shifted to using outcome-based measures to assess the impacts of management and resource provision. Outcome-based measures include animal health/physical condition and behavior (animal-based measures), as well as environmental quality. While the inclusion of outcome-based measures in standards and audits has a range of benefits, there are a number of important considerations and constraints related to the use of such measures. This presentation will address those considerations and constraints, including the selection of measures and the development of meaningful and practical scoring systems, as well as the determination of benchmarks appropriate for different housing systems, regions, genetic backgrounds, and changes in behavior and health status that normally take place during animals' lifetimes. A significant constraint is that behavioral outcomes can be very difficult to measure *in situ* because of the time and expertise needed for data collection. Some behaviors can be evaluated by using health/physical condition proxies (e.g., injury due to aggression). For other behaviors, however, the best way to improve outcomes may be by specifying the type and quantity of resources needed to promote the performance of those behaviors, whether those resources are enrichments, space or social companions. Incorporating outcome-based measures also necessitates thinking differently about the goal (as well as public and user perceptions) of third-party auditing and inspection. Rather than "checking a box" that required resources are present, evaluators instead need to assess the appropriateness and effectiveness of corrective actions. Given that the causes of animal welfare problems are often complex, there may be a long timeframe before corrective actions can be identified that are successful in improving outcomes. Because of these kinds of challenges and constraints, balancing resource-based and outcome-based measures to ensure that standards and audits are meaningful requires clarifying both sector-specific animal welfare issues and program goals.

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EXPLORING OXIDATIVE STRESS AS A POTENTIAL INDICATOR OF ANIMAL WELFARE

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Glucocorticoids (GCs) are often used as a physiological indicator of animal welfare but can be challenging to interpret because of their primary metabolic role and potential to increase following positive events. New physiological indicators are needed, and targets related to oxidative stress, which are becoming common in conservation physiology, have promise. Reactive oxygen species (ROS) or “free radicals” are normal byproducts of cellular metabolism that are highly reactive and can damage proteins, lipids, and DNA. Normally, antioxidant defenses effectively neutralize ROS. Oxidative stress and subsequent damage occur when this balance cannot be maintained, either due to a lack of antioxidant capacity or an increase in conditions that promote ROS formation—such as elevated GCs. Therefore, measures of oxidative stress may be informative about the net biological costs organisms experience from stress after protective factors in other welfare domains (e.g., nutrition, social support) are taken into account.

At the Detroit Zoological Society, we have begun exploring oxidative damage to DNA as a welfare indicator. The rate of DNA damage caused by oxidative stress can be measured via enzyme-immunoassays that target oxidized guanine, including 8-hydroxy-2′deoxyguanosine (8-OHdG). We measured 8-OHdG non-invasively in feces from n=3 grizzly bears (*Ursus arctos horribilis*) and n=2 white rhinoceroses (*Ceratotherium simum*). In grizzly bears, we found that group log-transformed concentrations of 8-OHdG increased along with log-transformed fecal glucocorticoid metabolites (FGM) ($r=0.49$, $p<0.001$). On days with more affiliative behavior, log(8-OHdG) showed trends to decrease for one bear ($r=-0.42$, $p=0.096$) but increase for another ($r=0.44$, $p=0.056$), with the third showing no relationship. Results comparing log(8-OHdG) in rhinos during and after a zoo closure were also inconsistent. Only one rhino showed a relationship between log(FGM) and log(8-OHdG), with higher log(8-OHdG) related to lower log(FGM) ($r=-0.39$, $p=0.02$). The other rhino had higher log(FGM) when the Zoo was open than closed ($r=0.40$, $p=0.02$) and on days with more visitors ($r=0.59$, $p=0.003$). However, he showed no differences in log(8-OHdG), except for an increase with spending more time alert ($r=0.43$, $p=0.04$). Together, these data hint that 8-OHdG changes in response to welfare parameters, but it is difficult to interpret whether these idiosyncratic results are due to the small sample sizes, individual differences in oxidative physiology, or measurement techniques. Many validation factors require further exploration for fecal measurement of 8-OHdG, such as dietary effects. However, we hope these preliminary investigations encourage others to explore how biomarkers of oxidative stress may serve as indicators of animal welfare.

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NON-TARGET INTERACTIONS AND HUMANENESS EVALUATION OF CAPTIVE BOLT TRAP IN RODENT CONTROL

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Alternatives to rodenticides are desirable to pest control customers and operators concerned with the environment and animal welfare, but there are few practical alternatives to handle rodent infestations. The Goodnature® A24 automatic rat trap is a self-resetting captive bolt trap powered by pressurized CO₂, designed with the objective of instantly crushing the skull to cause spontaneous nervous system suppression. Conservation and environmentally-minded institutions that conduct rodent control are interested in these traps, but have concerns about the potential to harm non-target animals including other small mammals, birds, and pets. This study aimed to identify potential risks to non-target animals, and conduct a humaneness evaluation of the traps. Nine traps were deployed at a compost facility where rodents were present, and each trap paired with a motion-activated video camera. To assess non-target risk, the traps were baited, but not activated, and a cross-over design was used with a blocking device (“blocker”) to evaluate the effectiveness of this tool at reducing non-target strikes. A total of 2,282 animals across 34 different species were recorded by video camera. Rodents were the most common mammalian visitor (n=566), followed by squirrels (n=302), and other mammals (raccoons, skunk, shrew, n=92). Birds were the most frequently observed (n=1,312), but had the fewest interactions with the trap itself – only 1 bird was observed entering the trap body (with no blocker equipped), while the remaining interactions involved no interaction (n=1,087), investigating the trap (n=147) or perching on the trap (n=97). A small number of unidentifiable animals (n=10) were observed, but did not interact with the trap. 36 raccoons and squirrels were observed either entering the trap or inserting limbs in the trap when there was no blocker in place, however this number was reduced to 0 with a blocker in place. Once the researchers were confident in avoiding non-target strikes, the traps were activated and video footage observed to evaluate humaneness. To date, 11 animals have been observed activating the trap – 10 apparently instant kills. The Goodnature A24 rodent trap is a promising tool as an alternative to rodenticide poisons, however the researchers recommend the use of a blocker in outdoor settings where other non-target animals are present.

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NEWBORN CANNIBALISM IN LABORATORY MOUSE AND HOW IT BIASES THE ESTIMATION OF PUP MORTALITY

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Perinatal mortality is a major welfare and economic issue in laboratory mouse breeding. Previous studies suggested that newborn pup mortality could be underestimated, especially if counting failed to include pups cannibalised overnight. We compared two counting methods (daily cage checking alone (DAILY_CHECK, Nlitters=138) or in combination with video analysis (VIDEO_TRACK, Nlitters=55)) for estimating the number of C57BL/6 pups born, died and weaned. Studied litters were from trios with (TRIO-OVERLAP, Nlitters=90) or without (TRIO-NO_OVERLAP, Nlitters=103) the presence of another litter in the cage. Counting methods were compared using linear mixed models at the litter level. To further understand divergences between counting methods, we investigated pup mortality and cannibalisms (eating a dead pup) across time from video captured in VIDEO_TRACK counting in litters from TRIO-OVERLAP (Npups=170), TRIO-NO_OVERLAP (Npups=308) and SOLO (single dam, Npups=472). Kaplan-Meier method and logistic regressions were performed at the pup level. DAILY_CHECK counting led to a litter size underestimation of 35% compared to VIDEO_TRACK counting ($P < 0.0001$, **Fig.1**), while the estimated number of weaned pups did not differ between counting methods ($P > 0.10$). DAILY_CHECK counting estimated pup mortality at 2.0[1.8;2.4] dead pups/litter counted, less than half the estimate of 4.2[3.5;5.0] dead pups/litter in VIDEO_TRACK counting ($P < 0.0001$). Estimated numbers of pups born and died were lower in TRIO-OVERLAP than in TRIO-NO_OVERLAP litters ($P = 0.050$ and $P < 0.0001$). Although higher in TRIO-OVERLAP litters, analyses of VIDEO_TRACK data indicated that pup mortality was highest within the two first days of life for all litters. When exploring pup mortality between birth and first cage checking in d0, a total of 69%, 53% and 21% of dead pups were already cannibalised in TRIO-OVERLAP, TRIO-NO_OVERLAP and SOLO litters, respectively. Therefore, dead pups were 2.5 and 1.7 more likely to be cannibalised when born in TRIO-OVERLAP or TRIO-NO_OVERLAP than in SOLO litters ($P < 0.0001$ and $P = 0.007$). Pups cannibalised before the first daily cage check could only be detected later using video analyses. Infanticide (killing a live pup) was only observed 7 times in trios, meaning that most of the cannibalised pups were not actively killed before being eaten. These results show that (i) the number of mouse pups born and dead may be highly underestimated when cages are checked once every day and newborns counted at d0, (ii) dead pups eaten between birth and first cage checking is the main cause of this underestimate and (iii) the underestimate is greater for litters born when another litter is already present.

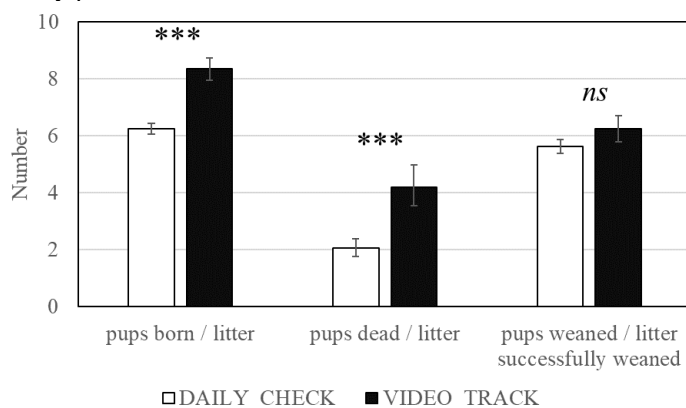


Figure 1. Mean (\pm SEM) number of pups born/litter, predicted mean [CI] number of pups dead/litter and mean (\pm SEM) number of pups weaned/litter successfully weaned (i.e., pups from complete litter loss excluded), counted using the routine daily check counting only (DAILY_CHECK, white columns) or combined with video analysis (VIDEO_TRACK, black columns). *** $P < 0.0001$.

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**CONSIDERING ANIMAL SPACE USE TO EVALUATE AND ENHANCE WELFARE:
EXAMPLES FROM THE ZOO ENVIRONMENT**

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Generally speaking, animals will spend time in environments in which they feel comfortable and avoid environments in which they feel less comfortable. This generalization allows us to gain insight into animal welfare, and how we can enhance it, by evaluating how animals use their space. At Lincoln Park Zoo, trained observers regularly record space use with the ZooMonitor app, which documents animals' locations within their habitat on an x, y-coordinate system. Here, we share insights gained and actions taken to enhance welfare for several species and contexts, including (1) domestic chickens (*Gallus gallus*) involved in a visitor-interaction program, (2) Sichuan takins (*Budorcas taxicolor tibetana*) exposed to varying shade availability, and (3) five species of primates observed with and without visitors present (black and white colobus, *Colobus guereza*, Allen's swamp monkeys, *Allenopithecus nigroviridis*, Bolivian grey titi monkeys, *Callicebus donacophilus*, crowned lemurs, *Eulemur coronatus*, and DeBrazza's monkeys, *Cercopithecus neglectus*). By quantifying and visualizing how space use changed across conditions, we were able to make inferences about internal states and generate action plans aimed at enhancing welfare. In sum, measuring space use is a simple and powerful way to make inferences about animal welfare across a broad range of species and environments.

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THINKING OUTSIDE THE LAB; CAN STUDIES OF PET RATS INFORM LABORATORY RAT WELFARE?

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The welfare of rats used in scientific research is an important concern; in the European Union alone, over one million rats were used in scientific procedures in 2017. An improved understanding of how to measure and enhance the welfare of rats is thus an important goal. However, studying the welfare of laboratory rats has, to date, only involved studies within the laboratory setting, leading to two potential pitfalls: (1) using laboratory rats to study their welfare, which can involve negative manipulations and ultimately euthanasia, poses an ethical predicament; and (2) the highly controlled housing conditions of many laboratories may mean that results are not generalizable to rats in other laboratories or from other suppliers. To this end, we investigated whether pet rats could provide an alternative and large study population to inform laboratory rat welfare. To achieve this, we conducted a survey of pet rat owners in which we asked questions about the conditions, behaviour, and health of their animals. In total, there were 677 respondents (who completed the survey in full) owning a total of 3893 rats. With regard to the welfare of laboratory rats, the study firstly identified a number of well-established and intuitive findings that supported the validity of this approach; greater play in younger animals, greater aggression in less-handled animals, and greater health issues in older animals. The study also demonstrated the importance of being able to express natural behaviours to the health of the animals; rats in more restrictive conditions were significantly more likely to exhibit respiratory issues, tumours, hind-leg degeneration, and cardiac problems. Importantly, the study revealed novel and potential markers of more positive welfare – namely ‘boggling’, which is the rapid and rhythmic bulging of the eyes resulting from movement of the rats’ masseter muscle (particularly in older rats), digging (particularly in female rats), and play (particular in young rats); the frequency of each of these behaviours was significantly reduced by greater exposure to predatory species, such as cats within the home. The study of pet rats may therefore provide useful and fruitful data to inform laboratory rat welfare.

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THE IMPACT OF VISITOR EFFECT ON JAPANESE MACAQUES' COGNITIVE PERFORMANCE AND WELFARE

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At Lincoln Park Zoo, macaques voluntarily participate in touchscreen testing. This research helps further the zoo's mission of public education and enhancing animal welfare. While providing a valuable source of enrichment for our macaques, cognitive research takes place in view of visitors, and crowd activity can be variable. Previous research has explored the impacts of visitors on zoo animals' behavior and welfare, but less work has considered how zoo visitors may influence animals' decision making, which may reflect affective states. So, in a pilot study, we evaluated potential visitor effects on four zoo-housed Japanese macaques' (*Macaca fuscata*) cognitive performance on a match-to-sample task. Macaques were tested in glass-sided booths next to a visitor viewing area. For every 12 trials completed, we recorded visitor numbers in the viewing area, coding crowds as small (0-20), medium (21-40), and large (41-60). We analyzed the macaques' response latencies and trial accuracy by crowd size and study period (the first three months vs. the second three months). We predicted that decreasing accuracy and increasing latencies with crowd size would indicate attention bias effects. In the first period, the macaques performed at chance levels and their accuracy did not differ by crowd size. In the second period, their accuracy significantly improved (as compared to the first period) but was mediated by crowd size. The macaques were significantly more accurate when small rather than when medium or large crowds were present. Latencies varied by study period and crowd size, with no evidence of a response-slowing effect. To further explore preliminary findings, we conducted a follow-up study with nine macaques, collecting data both when the zoo was closed due to COVID-19 restrictions (10 test sessions) and when the zoo reopened (10 test sessions). We again used a match-to-sample paradigm, but with different stimuli. Along with increasing our sample size, we recorded more detailed visitor variables including 1) visitor count, 2) visitor location within the viewing shelter, and 3) ambient sound levels. We found no difference in the monkeys' accuracy by presence of visitors (zoo open or closed) or by number of visitors when the zoo was open. Response times decreased when the zoo was open and varied across visitor number. Moreover, prior experience with the task was a better predictor of success than visitor-mediated variables. Thus, we believe that this voluntary research program simultaneously meets animal welfare and research goals.

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MATERNAL DEPRIVATION AFFECTS GOAT KIDS' STRESS COPING BEHAVIOUR

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Maternal deprivation early in life has been shown to disrupt neonates' development, but is a common practice in dairy husbandry by separating the young animals from their dams and other adults soon after birth. The implications of this partial social deprivation for animal welfare remains poorly understood, particularly for dairy goats. This study investigated the effects of partial social deprivation on goat kids' stress coping abilities. Twenty female kids were raised together with their dam (dam-reared) in a herd composed of other lactating goats and kids, while twenty female kids were separated from their dam three days after birth and reared together with same age peers (artificially-reared), visually separated from the lactating herd. All kids shared the same father and two thirds of the kids were twins allocated to each treatment. At one month of age, kids were individually submitted to a series of behavioural tests: a novel arena test, a novel goat test, and a novel object test. These tests happened consecutively in this order, and lasted 180 seconds each. The kids' behaviour was video-recorded and analysed *post-hoc* by an observer blind to treatments. Five weeks after weaning, the kids were also subjected to human-animal relationship tests. During the three behavioural tests, artificially-reared kids spent significantly more time rearing and vocalized, jumped, and ran significantly more often than dam-reared kids, but self-groomed and urinated significantly less often than dam-reared kids. During the novel goat test and the novel object test, artificially-reared kids gazed at the novel goat and the novel object significantly less often and initiated contact significantly more quickly with the novel goat and the novel object than dam-reared kids. Kids from either treatments however did not significantly differ in their salivary cortisol response to the tests. Artificially-reared kids also showed significantly less avoidance and more approach toward humans than dam-reared kids during the human-animal relationship tests. The higher intensity of their behavioural reaction showed that artificially-reared kids react to stressful situations more actively than dam-reared kids. The difference between the three tests were only minor, suggesting a general change in the kids' response to stressful situations rather than a specific change in their social response, tested with an unfamiliar adult. Hence, artificial rearing affects goat kids' behavioural response to challenges, probably maternal deprivation being the main factor. Further research should investigate its welfare implications such as for stress resilience, and its potential long-term impact on the animals.

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DOLPHINS' "WILLINGNESS TO PARTICIPATE" (WTP) IN POSITIVE REINFORCEMENT TRAINING PREDICTS EARLY CHANGES IN HEALTH STATUS

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Despite significant advances over the last decade, welfare scientists continue to search for the holy grail: measures of overall welfare state that are both practical *and* valid. An animal's motivation to acquire certain rewards in its environment has underpinned many existing welfare indicators, such as preference tests, cognitive bias and anticipatory behaviour, but linking these measures to the animal's physiology and health has proved difficult. Bottlenose dolphins (*Tursiops truncatus*) tend to mask symptoms of poor health, and their management in captivity would benefit from the correlation of health and behavioural parameters. In this study, four representative behavioural and health measures were chosen: health status (as qualified by veterinarians), percentage of daily food eaten, occurrences of new rake marks (proxy measure of social activity), and "Willingness to Participate" (WtP) in positive reinforcement training sessions, measured qualitatively by their caretakers. These data were collected over a year and multiple times a day, every day from a large sample size ($N_{\text{dolphins}} = 51$). First, it was found that dolphins with a higher WtP score also had a significantly better health status, ate a higher percentage of their daily food, and a lower occurrence of new rake marks. In addition, the WtP score was found to be significantly lower up to 3 days before a reduced health state was diagnosed; however, the percentage of daily food eaten and new rake mark measures did not show any significant change before such a diagnosis. These results suggest that measuring WtP in training sessions is a potential behavioural measure of dolphin welfare, given its correlation to each of the three other behaviour and health measures. We found WtP to be a sensitive indicator of changes in the dolphins' health state and suggest its utility in veterinary care and general management as a practical and non-invasive tool. Our findings suggest more work is needed to reveal the influence of dolphin social behaviour on health, but we propose WtP as a behavioural measure that is both practical and valid for dolphin welfare assessment.

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SHOW ME THE BRACHY DATA! ARE FLAT-FACED DOGS REALLY UNHEALTHIER THAN OTHER DOGS?

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Created by mankind over a century ago for specific working purposes, brachycephalic (flat-faced) dog breeds are now commonplace in the general dog population but generally are kept as pets these days. These breeds often inspire strong breed loyalty from their owners, with “cute” brachycephalic doggy-faces making many humans smile involuntarily. However, mounting evidence on their health problems are challenging the intrinsic welfare acceptability of the brachycephalic phenotype. But where is the truth here? Even if they are predisposed to some disorders, maybe they are protected (i.e. at lower risk) to other disorders that counterbalance the welfare costs? Is ‘brachy bashing’ just fake news in a world addicted to the allure of hyperbole?

Assessment of overall health status offers objective metrics that can support inferential welfare comparison. Using anonymised veterinary clinical records, this study aimed to characterise and compare the prevalence of common disorders between brachycephalic and non-brachycephalic dogs. Review of the differing predispositions and protections between these groups would contribute to a deeper understanding of the overall health polarity of brachycephalic dogs.

The study included a random sample of 22,333 dogs of all types from 955,554 dogs under UK veterinary care within the VetCompass Programme. VetCompass clinical records were manually examined to extract data on all disorders recorded during 2016. Multivariable binary logistic regression examined brachycephaly as a risk factor for each of a series of common disorders.

Compared to other dogs, brachycephalic dogs were generally younger, lighter and less likely to be neutered. This suggests that much of the older literature that relied on univariable analysis may be unreliable due to confounding bias. The same four disorders (periodontal disease, otitis externa, obesity and anal sac impaction) featured in the top four disorders for both brachycephalic and non-brachycephalic dogs, suggesting some important health similarities between both groups. However, after applying multivariable methods, the health status of brachycephalic and non-brachycephalic dogs differed for 10/30 common disorders. Of these, brachycephalic types were predisposed for eight disorders (e.g. corneal ulceration x8.4 risk, umbilical hernia x3.2 risk) and were protected for two disorders (undesirable behaviour x0.6 risk, claw injury x0.5 risk), suggesting that the health of brachycephalic dogs was poorer overall than non-brachycephalic dogs.

This presentation will explore similarities and differences in health status between brachycephalic and non-brachycephalic dogs and aim to reach some conclusions and inference that can support efforts to improve canine welfare in relation to extreme conformations, and especially brachycephaly.

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TICK CONTROL IN THE BOVINE POPULATION OF PAKISTAN: A STEP TOWARDS ANIMAL WELFARE

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Ectoparasites are ubiquitous, often causing severe threats to livestock and, in most cases, challenging to eradicate permanently; hence, they are usually managed at a local level with continuous use of the similar insecticides or endectocides. Ectoparasites cause not only economic losses of 13.8 to 18.7 US billion-dollar globally but also create drastic concerns toward animal health and welfare, morbidity and mortality - due to their potential blood-sucking nature. Their increasing resistance and lower drug efficacy demand more sophisticated and integrated approaches to reduce the losses associated with such arthropod pests. Among ectoparasites, ticks remained the major concern in the livestock sector of Pakistan. There are three main tick-borne diseases (TBDs); babesiosis, theileriosis and anaplasmosis in Pakistan, in addition with a fourth known as cowdriosis (heartwater) causing great concern regarding animal health around the globe. For tropical theileriosis, mortality rates vary from 90 % (in exotic breeds) to 5 % or less (in indigenous breeds), while 90% mortality has been reported in case of untreated babesiosis in Pakistan. As dairy sector of Pakistan is developing through imported breeds of Holstein Friesian, tick burden is great animal health and farm economic concern. According to a study, theileriosis causes US \$74.98 per animal and 13.83% of total Holstein Friesian dairy farm costs. More than 80% of the cattle populations are exposed to ticks and responsible for the transmission of various TBDs in Pakistan. Tick infested animals face the loss of blood and blood protein, debilitation, depression of immune function, damages to skin, hides and decrease in their productivity. Tick infestation also disturbs the animal resilience through stress, irritation, reduction in grazing and rumination time and even death in severe cases. These major problems for the bovine population; not only affect the animal health, survival and well-being but also raised animal welfare concerns due to treatment failures. This study emphasizes the importance of prevention rather than cure through proper biosecurity measures to minimize the reintroduction of the infestation once they have been eliminated and use adapted drugs. Moreover, molecular techniques, simulation modelling and particularly the use of satellite imagery, the use of resilient livestock breeds and a fuller understanding of the effects of better nutrition, may also all make important positive contributions towards an integrated animal welfare.

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TRACKING STRESS REMOTELY VIA WOOL HORMONES AND SMART TAGS: SHEARING EARLY IN PREGNANCY REDUCES STRESS AND IMPROVES PRODUCTIVITY OF AUSTRALIAN MERINO SHEEP

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Farm animal productivity continues to be significantly boosted by new animal welfare technology that can track animal behaviour and physiology. One of the key monitoring knowledge gaps within domestic livestock (sheep) research is maternal grazing behaviour and wool productivity growth. Each of these areas are influenced by environmental and on farm management factors. In this novel research, we applied behaviour and endocrine physiology monitoring tools to assess whether a simple management change of shearing frequency through pregnancy could influence the performance and productivity of Australian Merino sheep. We conducted a field research in New South Wales, Australia using a cohort of $n = 48$ Merino ewe sheep randomly selected from a group of 100 pregnancy scanned ewes. All ewes were shorn in summer of 2018 and naturally joined to rams in early 2019 for four weeks. Those ewes that did not fall pregnant were removed from the trial at pregnancy scanning. Those ewes that had pregnant confirmed status were randomly assigned to two groups of $n = 24$ ewe sheep. All ewes were tagged with a SMART collar sensor capable of recognizing the grazing activity of ewes *via* satellite and cloud based remote monitoring system. We also applied wool glucocorticoid (stress hormone) technique to index absolute levels of cortisol in ewe sheep early, mid and late pregnancy. One group of pregnant ewes were shorn early while the second group remained unshorn throughout gestation. In addition, we applied a novel epigenetic method (DNA methylation profiling) to determine if shearing frequency influenced DNA epigenetics in the ewes and lambs. Productivity was assessed by quantifying the wool quality indices of winter born lambs from both cohort of ewes. Results of the shearing experiment showed that ewes that were shorn early in gestation improved their grazing activity by 10%. Body condition scores (BCS) of the twice shorn ewe when compared to pregnant ewes that were carrying full fleece increased by 0.1 of mean BCS ($p < 0.05$). Ewes shorn early in pregnancy also had significantly lower mean levels of wool cortisol ($p < 0.05$) in late gestation compared to ewes that remained unshorn. Wool productivity results showed the lambs born from early shorn ewes had produced finer wool as well as wool showing improved comfort factor and spin fineness. The results of the epigenetic DNA methylation testing were non-significant between the shearing frequency treatments. Collectively, the results from this research demonstrates the powerful technology capability that can be used to monitor the behaviour and physiology of sheep on farm and the dataset can be used to assess the performance and productivity levels of ewes in relation to management and environmental factors. It is hoped that the technology of wool hormone and SMART tags monitoring tools can be adopted by farmers to index stress levels of sheep and help to better monitor sheep welfare.

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PREDICTORS OF PSYCHOLOGICAL STRESS AND BEHAVIOURAL DIVERSITY AMONG CAPTIVE RED PANDA IN NORTH-EASTERN INDIA AND THEIR IMPLICATIONS FOR GLOBAL CAPTIVE MANAGEMENT

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At high altitudes, biodiversity is at elevated risk from extinctions due to rapid environmental changes. In the eastern Himalayas, the red panda, an endangered species, is struggling to survive in the wild, and a global captive breeding programme has been launched to conserve the species; three Indian zoos ($n=26$) participate in this programme, and all are located within the red panda's natural range. Because captivity can have negative impacts on animals, reducing the chance of successful reintroduction, we investigated the predictors of stereotyped behaviour and behavioural diversity in these zoos. Stereotypy decreased with quantity of bamboo supplied, density of logs and trees, and number of nests provided. Behavioural diversity decreased with number of visitors, frequency of feed, ambient temperature, number of nests, and among males compared to females; it was greater when the pandas were managed in pairs rather than alone. However, there were differences in both stereotypy and behavioural diversity among the three zoos. Provision of more nests was associated with lower stereotypy in the pandas, but also with lower behavioural diversity; this may be because inactivity during day time is natural behaviour in this nocturnal species. Captive management should aim to provide an environment conducive to natural behaviour. Findings from this study have global relevance, as the red panda experiences similar welfare issues in captive systems around the world. We suggest feeding the red pandas with adequate bamboo, and enriching their enclosure with a large number of trees, logs and nests to reduce stereotypy and support natural behaviours.

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EFFECTS OF HATCHING SYSTEM ON BROILER ACTIVITY MEASURED INDIVIDUALLY BY AN ULTRA-WIDEBAND TRACKING SYSTEM

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Broilers usually hatch in hatcheries without access to feed and water until placement at the farm, which can affect their health, resilience and welfare. Therefore, alternative concepts have been developed, such as providing chickens with early nutrition in the hatchery or hatching eggs on-farm. However, information on the resilience and welfare of chickens hatched in these systems is limited. Changes in broiler activity are a promising indicator for various resilience and welfare threats. Sickness and lameness for instance have been associated with lower activity levels in broilers. With modern sensor technology, it is possible to automatically record group- but also individual activity patterns. The aim of this study was to assess the effects of hatching system on broiler activity in general and after the challenging situation of vaccination.

Therefore, a proof-of-principle-experiment was performed with chickens that hatched either conventionally in the hatchery (HH), in a system which provided feed and water in the hatcher (HF, hatchery-fed) or on-farm (OH). The animals were reared in 3 floor pens (one pen/hatching system, 30 animals/pen) in a room, in which an ultra-wideband (UWB) tracking system was installed. At d14, active tags of the UWB system were attached to 5 chickens/pen. The distances moved (DM) by the birds were tracked for 4h/d until d34. At d28, all chickens were vaccinated individually with a live vaccine against Infectious Bronchitis.

There was a significant age x hatching system interaction for the DM by the chickens ($F_{2,306} = 5.57$, $P < 0.001$). During the 3rd week of life, HH chickens moved less than HF and OH chickens, whereas there was no difference between HF and OH chickens in week 3, and between all three groups in week 4 and 5. Within all groups, DM differed between individual chickens (HH: $F_{4,90} = 46.42$, $P < 0.001$; HF: $F_{4,90} = 48.50$, $P < 0.001$; OH: $F_{4,90} = 80.46$, $P < 0.001$). To analyse the effects of the vaccination challenge, DM were tested for within-subject factors (DM on 3 days before and after the challenge, respectively) and between-subject factors (hatching system). DM did not differ within subjects. However, there was an effect of between-subject factors for the three days following the challenge, with HH chickens moving longer distances than HF and OH chickens ($P < 0.001$).

The results indicate that hatching system affected broiler activity at specific ages and days after a vaccination challenge, with chickens from two alternative systems showing similar activity patterns. Within all 3 hatching systems, activity also differed among individual birds.

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**DEVELOPMENT OF TOOLS FOR FARMERS TO SELF-ASSESS THE WELFARE OF THEIR
POULTRY AND PIGS IN ORGANIC AND OUTDOOR SYSTEMS**

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A goal of the PPILOW project is to develop and evaluate tools for animal welfare self-assessments by poultry and pig farmers of organic and outdoor systems. To accomplish this goal, two smartphone applications are being modified or developed in eight different languages. The EBENE[®] app, which was primarily developed for conventional poultry farms, was adapted to be more suitable for outdoor farming systems and the PIGLOW[®] app for outdoor pig farms was newly developed.

For both apps, a selection of welfare indicators to be included in the assessments was made based on a suitability analysis of indicators from existing welfare monitoring protocols (e.g. Welfare Quality[®], Dierenwelzijnscan). The opinions of National Practitioner Groups (NPGs), including representatives of feed producers, consumer associations, retailers, veterinarians, processors and farmers, were taken into account in this decision process. NPGs from six and three countries were surveyed for EBENE[®] and PIGLOW[®], respectively. Preference was given to animal-based indicators, all of which fit into one of the four welfare principles of the Welfare Quality[®] protocols, namely good feeding, good housing, good health and appropriate behaviour. A trade-off was made between the time investment by the farmers and the level of detail of the assessment, resulting in assessments that take approximately one hour to complete. The results of the apps include scores for all welfare indicators and anonymous benchmarking. The farmers also receive automated feedback for each welfare indicator that includes an explanation of and risk factors for related potential welfare problems. Separate welfare assessments are available for broilers and laying hens, and for fattening pigs and sows at different production stages. Additionally, both apps have the possibility to assess the depopulation process. The user-friendliness and feasibility of the apps was tested during on-farm trials in Belgium and France, after which minor adjustments were made to wording and specific questions.

The use of these tools for welfare self-assessments could sensitize farmers to the presence of potential welfare problems and the automated feedback could motivate and guide them to take corrective actions or seek additional advice of experts. A longitudinal study to assess the effect of the use of the apps on animal welfare and the farmers' opinions of the apps is currently being conducted on commercial fattening pig and broiler farms.

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BEHAVIOURAL, PHYSIOLOGICAL AND PATHOLOGICAL ASSESSMENT OF DECOMPRESSION PARAMETERS FOR THE POTENTIALLY HUMANE KILLING OF ANAESTHETISED LABORATORY RODENTS

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Rodents are the most widely used laboratory animals worldwide, with millions used annually. The vast majority of these are killed either during or after the scientific work and, according to current legislation, must be killed humanely. Gradual exposure to carbon dioxide (CO₂) remains the most common method of killing laboratory rodents, despite considerable evidence demonstrating aversion, including induction of anxiety, dyspnoea, and pain at high concentrations. There is an urgent need to find a humane, practical, and high-throughput alternative.

We are systematically investigating whether hypobaric hypoxia (via gradual decompression, a process equivalent to ascending to high altitude) could be a novel and humane approach to the killing of laboratory rodents. Hypobaric hypoxia occurs at low atmospheric pressures due to a proportional decrease in the partial pressure of oxygen, and exposure to these environments ultimately leads to unconsciousness and death. Previous welfare assessments have shown that gradual decompression is humane for use in chickens.

The goal of this trial was to assess the effectiveness of hypobaric hypoxia as a killing method for mice. We investigated the effects of both decompression rate and curve profile shape (accelerated, gradual or linear). We characterised the behavioural, physiological, and pathological consequences of each decompression profile in anaesthetised mice in a custom-made decompression chamber.

We found significant effects of decompression rate irrespective of profile shape on several behavioural measures. Compared to the faster 150ms⁻¹ rate, we found a lower rate of gasping with the slower 75ms⁻¹ rate, as well as increased latencies to first gasp, agonal gasping, cessation of rhythmic breathing and time to motionless/death. Although we found no independent effect of profile shape, decompression rate effects were apparent in the linear and gradual profiles only, highlighting that the accelerated profile shape is least susceptible to meaningful refinement via rate. Overall, we found no concerning pathological changes in the gut and most other body areas examined. While lung haemorrhage and congestion were seen, this was less pronounced than with CO₂ killing. Ear pathology presented some cause for concern, with most mice exhibiting moderate middle ear congestion and haemorrhage, but partial rupture of the tympanic membrane was seen in only one mouse.

We discuss our findings in relation to the effectiveness of hypobaric hypoxia to kill laboratory rodents and to do so without pathological damage that might result in negative conscious experiences, and outline subsequent work comprehensively exploring the welfare impact of gradual decompression in conscious mice.

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DEVELOPING AND VALIDATING ATTENTION BIAS TOOLS FOR ASSESSING TRAIT AND STATE AFFECT IN ANIMALS: A WORKED EXAMPLE WITH *MACACA MULATTA*

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Attention bias is a new approach to assessing animal affect that has shown promising results in several animal species. It describes a tendency to preferentially attend to emotional compared to neutral cues and is influenced by underlying affect. It is important in the early days of this new field that we develop widely utilisable methods and incorporate lessons from the human literature from which tasks are adapted. This fundamental knowledge is critical to the development of standardised and sensitive tools, and the validation of experimental protocols to ensure best practice. Here, we describe protocols for two preferential-looking attention bias tasks. Study 1 involved a manual task using freely available low-cost materials. Study 2 used an automated task requiring specialist equipment and programming, but presumably less prone to noisy data. Tasks were tested with 109 socially housed rhesus macaques, *Macaca mulatta*, who had been trained to sit by a target, but received no other training. Tasks involved showing animals emotional face pairs (threat-neutral), and subsequent blind coding of video for duration of looking at either face. Three measures of social attention were examined: time spent looking at the threat face (THR), total time looking at the threat-neutral face pair overall (TL), and attention bias difference score (ABD) calculated as time spent looking at the neutral face subtracted from time spent looking at the threat face. Based on the human literature and early primate work, the influence of five potential confounding factors on attention was assessed: trial number, stimulus ID, previous testing experience, time of day and visual field to which the threat face was presented; as were several life history factors: sex, age, and social rank. Both tasks revealed stable individual differences in baseline social attention (THR and TL: effect sizes=0.15-0.31; repeatabilities =0.12-0.26; suggesting sensitivity to trait affect), but not ABD (which may be more sensitive to brief shifts in emotion state). All potential confounding factors had a significant effect on at least one measure of social attention. For a subset of monkeys who took part in both Study 1 and Study 2 several years apart (n=18), there was significant reproducibility between tasks for all three measures (R=0.15-0.63), supporting an argument for stable individual differences in baseline attention bias, and validating the two tasks for measuring the same trait. The attention bias method shows promise for further development of standardised protocols with animals. We provide framework and recommendations for future method development.

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BENEFITS OF A SOCIALISATION PROTOCOL ON COMMERCIALY BRED DOGS

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To meet the growing demand, dogs are increasingly being bred in large-scale commercial breeding establishments (CBEs). However, there is mounting evidence that puppies reared in these CBEs have limited exposure to people and novel stimuli affecting their development during the primary socialisation period.

We were granted access to a commercial breeding establishment (CBE), where we experimentally tested a novel socialisation protocol. We hypothesised that puppies exposed to the protocol would show less fearful behaviours towards novel objects and a novel person than those receiving socialisation efforts currently undertaken in the CBE. In the protocol we exposed 37 CBE puppies to a socialisation treatment between six and eight weeks of age and kept a control group of 39 puppies in baseline conditions. Litters were split with one half receiving the socialisation treatment and the other half only experiencing baseline conditions. The socialisation involved an escalating protocol of exposure to novel stimuli, for a total of five days. At eight weeks, all puppies were subjected to a battery of tests in a gridded arena involving novel objects and a novel person. Each test was filmed, and the videos analysed by a researcher blind to the socialisation treatment using BORIS video coding software.

Preliminary analysis has showed that socialised puppies were found to be more active; they crossed significantly more lines in the testing arena (Welch; $t = -4.18$, $df = 23.0$, $p = 0.0004$) and had a higher frequency of walk events (Welch; $t = -2.66$, $df = 22.5$, $p = 0.014$). They also spent significantly less time in the initial square of the testing arena (Wilcoxon; $W = 137$, $p = 0.0008$), had a decreased latency to approach a novel person (Wilcoxon; $W = 149$, $p = 1.73e-05$) and time spent with the novel person was higher in the socialised group (Welch; $t = -4.43$, $df = 20.9$, $p = 0.0002$). Socialised puppies had significantly longer durations of tail wagging (Welch; $t = -3.38$, $df = 22.1$, $p = 0.003$) and shorter durations of the tail being lowered (Wilcoxon; $W = 125$, $p = 0.011$), and they also had significantly shorter durations of a lowered posture (Wilcoxon; $W = 145$, $p = 0.0003$).

This study aimed to address current knowledge gaps regarding levels of socialisation required to produce less fearful dogs capable of coping in the family home. It is hoped that this research will help enable the creation of appropriate and practical socialisation protocols that will inform welfare enhancement programmes for dogs in a range of environments, including CBEs.

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FISH WELFARE EVALUATION INDEX BASED ON THE PREVALENCE AND SEVERITY OF EXTERNAL MORPHOLOGICAL DAMAGE

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Scientific studies regarding fish being sentient with cognitive capacities and pain perception, raised the need to quantify fish welfare accurately by implementing standard procedures for welfare assessment in aquaculture. Aim of this study was to define on-farm welfare indicators that are reliable, valid and practicable to set up a welfare evaluation index tested by the example of rainbow trout (*Oncorhynchus mykiss*) in commercial flow-through systems in Germany. Potential on-farm welfare indicators assessed on nine different farms included among others management (tank design, water supply and -exchange, stocking density, feeding frequency), water quality (temperature, oxygen, pH, nitrogen compounds), behaviour- and health observations (disease- and low welfare indicating behaviours, social interactions, schooling, activity level, crowding, external morphological damage), condition factor, somatic organ indices (SSI, HSI, CSI), macroscopic organ health (liver colour, gill health) and external morphological damage (skin-, fin- and eye injuries, emaciation, skeletal deformities). To gain additional in-depth insights into the effects caused by differences in management and water quality, various health and stress parameters (glucose, lactate, osmolality, histology, scale cortisol, molecular marker) were assessed and correlated to the on-farm welfare indicators. Management, water quality and behaviour correlated to external morphological damage, indicating that suboptimal rearing conditions potentially result in a visibly degraded external appearance of fish. Results further showed a positive correlation of histopathological changes as an indicator for health as well as scale cortisol as an indicator for chronic stress, in relation to external morphological damage. These findings demonstrate external morphological damage to be highly relevant welfare indicators. Although not less important, welfare indicators such as management, water quality and behaviour can be problematic in terms of reliability, validity or practicability depending on the rearing system. External morphological damage on the other hand can reliably be assessed by using photographic images that depict the different severity grades of impairment. Such method is easy and fast to learn and to apply, without incurring material costs. The welfare evaluation index summarizes the external morphological damage including severity and prevalence, resulting in categories of good, moderate and poor welfare. The welfare evaluation index can be employed by fish farmers, veterinarians and by monitoring- or certification programs to determine and control the state of fish welfare. The index serves as a first step in assessing the welfare state of fish, and can be followed by further measurements and adjustments to determine and eliminate the causes behind fish welfare impairments.

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**WHEN “ONE HEALTH, ONE WELFARE” ENCOUNTERS RABIES IN THE STREETS OF INDIA.
AN ANTHROPOLOGICAL PERSPECTIVE**

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Rabies is endemic in India, where 21,000 people (one-third of the human deaths caused by this disease worldwide) die of it every year. Dogs are the main reservoir, being responsible for 95% of these infections. The One Health approach is currently considered the best strategy to control rabies, so the WHO and the OIE recommend prompt vaccination of bitten individuals and mass vaccination of (free-roaming) dogs. Upon suggestion from the Animal Welfare Board of India, since 2001 India has been customising these guidelines. First and foremost, a paradigmatic shift has been necessary: the legally meaningful expression “street dog” was coined to replace the term “stray dog” and the underlying idea that free-roaming dogs, whether owned or unowned, are a problem to remove from the streets. According to the Animal Birth Control (Dogs) Rules of 2001, now street dogs have to be caught for vaccination *and* sterilization, and then they have to be brought back to their place on the street, where they have the right to live. Mainly because of the costs and the logistical challenges required by this strategy, these Rules are not providing the expected results either in controlling rabies or in reducing the street dog population. Inevitably, this failure enrages the public and fuels a heated debate on the very idea of One Health, but especially on its development into the One Health, One Welfare concept. For decades, people in India have been struggling to understand and accept the need for vaccinating dogs instead of continuing to kill them. Nowadays, they are also asking themselves whether having dogs living on the street, exposed to diseases, violence, accidents, neglect, and harsh competition over food and shelter, truly means taking care of their welfare. As with all health-related issues, culture shapes the definitions and mental representations of (animal) welfare that people may have, or may be willing to (re)negotiate. This is a key aspect in the issue under examination, as in the Indian cultural and religious context dogs have been traditionally suffering from severe neglect and scorn. This presentation will discuss the complexities involved in the framing of the concept of dog welfare in this peculiar setting, the challenges faced by India in its attempt to control canine rabies, and the debate on the possibility of “street dog welfare” being an oxymoron.

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ACUTE EFFECT OF FLUID CONTROL ON THE WELFARE OF LABORATORY RHESUS MACAQUES

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Rhesus macaques are widely used in biomedical research due to their phylogenetic proximity to humans and their ability to perform complex cognitive tasks. Some studies require a large number of trials and therefore a high motivation from the subjects. One way to achieve those is to control the amount of fluid available to the subjects in their home cage and to use fluid rewards during experimental tasks. Previous studies have shown that fluid control does not have a significant impact on the physiology of the animals. However, the potential psychological effect of this routine procedure is still debated. Indeed, limitations of previous studies, such as a small sample size or a sub-optimal control period, have complicated their interpretation. In this study, we aimed to investigate the acute effect of fluid control on macaques' welfare using a behavioural approach. Video-footage of 21 adult macaques (5 females and 16 males) was used for this study. Home cage behaviour of the animals was recorded in two conditions: when they had been fluid-controlled for at least 24 hours and when they had access to fluids ad libitum. The fluid control was part of ongoing experimental protocols unrelated to the present study. Animals' affective states were assessed by quantifying the frequency of (1) pharmacologically-validated behavioural indicators of anxiety (i.e. self-scratching, body shaking and self-grooming); and (2) behaviours suspected to indicate a negative acute affective state but still lacking proper validation (e.g. pacing, yawning, Inactive not alert behaviour). 504 hours of video footage were analysed, covering up to 5 years of intermittent fluid control per subject. Using generalised linear mixed models and a within-subject mean-centring approach, we disentangled the between- and within- subject effects of fluid control, and controlled for the effect of time. We found no linear effect of fluid control on the frequency of any behaviour nor any indication of habituation or sensitisation over time. Additionally, the number of consecutive days under fluid control within the same week had no effect on the indicators of negative welfare. Using an unprecedented number of animals, this study suggests that fluid control, as implemented at Newcastle University, does not have a negative impact on the acute affective states of macaques. Since fluid control protocols vary widely over the world, our results might encourage other research groups to implement the protocol used at Newcastle.

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ACCELEROMETERS AS TOOLS TO MONITOR BEHAVIOUR: A POTENTIAL REFINEMENT FOR THE WELFARE ASSESSMENT OF RHESUS MACAQUES USED IN NEUROSCIENCE RESEARCH

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Assessing welfare in rhesus macaques (*Macaca mulatta*) used in neuroscience research relies on obtaining physiological and behavioural measures to optimise and implement the 3Rs: Replacement, Reduction and Refinement. Through effective welfare assessment, potential refinements to laboratory practices may be highlighted.

Measuring behaviour and physical activity can offer key insight into animal welfare. Traditional methods of behavioural assessment are limited by resources and observer influence. We aim to quantify activity and behaviour using compact, uniaxial accelerometers. Accelerometers are placed inside individually designed soft, microfiber neck collars. This provides non-invasive, continuous measurements of activity in freely moving, group-housed, research macaques. Data has been collected intermittently over 5 years, when opportunity allowed, from a total of 47 individual macaques enrolled in long-term neuroscience studies at the University of Oxford. Non-invasive collar fitting and continuous data collection, in parallel with ongoing neuroscience protocols, provides opportunities to explore changes in macaque activity throughout their neuroscience careers. Accelerometers collect data before and after potentially welfare compromising events, e.g. neuroscientific procedures.

We investigate multiple analyses of this data to explore activity and behaviour. Preliminary analysis shows individual variation in activity budget; factors such as age, weight and social structure appear to contribute to individual activity patterns. Additionally, preliminary results show change in macaque activity in response to significant events. Numerous methods are explored to assess behaviour using this data. One approach, a knowledge-based method, classifies behaviour by comparing accelerometer and expert-scored video data (n; 4 different animals, 2-4 hours of video analysis per animal). This method differentiates between behavioural categories such as resting, foraging and locomotion. Other methods also explored, including logistic regression and Hidden Markov models, use exclusively accelerometer data.

Accelerometry is a powerful method, providing a non-intrusive, convenient measure of animal welfare in order to keep advancing the 3Rs.

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MACHINE LEARNING ALGORITHMS TO CLASSIFY AND QUANTIFY MULTIPLE BEHAVIOURS IN DAIRY CALVES USING A SENSOR: MOVING BEYOND CLASSIFICATION IN PRECISION LIVESTOCK

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Individualised sensor based behavioural monitoring in livestock offers increased sensitivity, objectivity and continuity that can help guide practices to improve health and welfare and detect ill health. However current technologies in calves only detect basic behaviours which limits their usefulness for health and welfare monitoring. Furthermore, some behaviours, such as locomotor play in calves, occur rarely. This means that simply counting the positive predictions of an algorithm can lead to vast over-estimation of the behaviour's prevalence. Here, we equipped thirteen pre-weaned dairy calves with collar-mounted sensors and recorded their behaviours using video cameras. Trained observers labelled behaviours according to a detailed ethogram. Sensor signals were merged with behavioural observations and signal features were calculated. We created an AdaBoost ensemble machine learning algorithm with 5-fold cross validation to classify labelled behaviours. In order to quantify low prevalence behaviours we developed an adjusted count quantification algorithm and predicted the prevalence of locomotor play behaviour on a test dataset with low true prevalence (0.27%). Our classification algorithm identified locomotor play (99.87% specificity, 98.98% sensitivity), self-grooming (98.90% specificity, 91.91% sensitivity), ruminating (96.84% specificity, 81.06% sensitivity), non-nutritive suckling (96.22% specificity, 87.76% sensitivity), nutritive suckling (98.49% specificity, 84.85% sensitivity), active lying (95.01% specificity, 64.14% sensitivity) and non-active lying (97.15% specificity, 88.89% sensitivity). Our results detail recommended sampling frequencies, feature selection and window size. Despite the low prevalence, our quantification algorithm's estimates of locomotor play behaviour were highly correlated with the true prevalence (0.97; $p < 0.001$) with a total overestimation of 18.97%. This is the first study in precision livestock farming to jointly tackle two machine learning tasks, multi-class behavioural classification and quantification of low prevalence behaviours. We believe this work will contribute towards behavioural insights and improve our ability to evaluate the health and welfare of calves.

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RUBBER NET MESH REDUCES SCALE LOSS IN FARMED ATLANTIC SALMON, *SALMO SALAR*

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Atlantic salmon, *Salmo salar*, are an economically and ecologically important fish species that interact with humans during farming, fishing, environmental monitoring and research operations. Routine handling in nets is an almost unavoidable consequence of these activities, exposing fish integument to net mesh. This can cause scale loss and subsequently exposes fish to pathogenic and osmoregulatory stressors.

To promote welfare and experimental refinement, a simple method was established to allow scale recovery, analysis and comparison, during netting and routine stock management. A study was performed in a controlled environment to investigate the effect of net mesh type (rubber coated or conventional knotless equivalent) and the number of fish per net (capture density) on scale loss for two size grades of salmon.

Up to 3 large adult salmon (mean 900 g) or up to 15 small smolts (mean 145 g) were briefly captured in hand-nets (both bag volumes ca. 7 L, mesh size 6 mm) during routine immersed stock movement between tanks, using a variety of circular transportation containers to allow at least 10 L volume per fish. After fish recovered and were removed, scales were collected and counted from containers, confirming that for both size grades scale loss was generally proportional to capture density.

For large adult salmon, scale loss significantly increased with capture density when knotless mesh was used, however the relationship was weaker and not statistically significant for adults handled in rubber mesh.

Small smolts also demonstrated significantly reduced scale loss when handled with rubber mesh compared to knotless mesh. Small smolts handled in knotless mesh also showed a more marked increase in scale loss, as capture density increased.

The scale recovery method suggests a low-tech and rapid approach to safeguard fish welfare, quantitatively compare net types and optimise husbandry techniques. This proof of principle study also suggests fundamental but simple improvements to fish handling and net design.

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REPRODUCIBILITY AND OPEN SCIENCE: LEARNING FROM PSYCHOLOGY

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Reproducibility failures have affected all parts of the life sciences relevant to animal welfare research, from animal behaviour to molecular biology. Psychologists have responded constructively and creatively to their field's very public reproducibility crisis. The solutions include new ways of doing experiments, such as Registered Reports and aspects of Open Science. To illustrate some of the challenges of traditional approaches to animal behaviour, relevant to animal welfare research, I will use the story of the "putative human pheromones" androstadienone and estratetraenol which, despite never having been shown to be pheromones, have been the subject of some 60 studies claiming 'significant' positive results. These are quite possibly false positives, part of the 'reproducibility crisis', sadly common in the rest of the life and biomedical sciences, which has many instances of whole fields based on false positives. Animal welfare research would benefit from vigorously adopting the proposals made by psychologists to enable better, more reliable science, with an emphasis on enhancing reproducibility. A key change is the adoption of study pre-registration and/or Registered Reports, which will also reduce publication bias. A growing number of journals covering animal welfare offer Registered Reports, including *Animals*, *Animal Behavior and Cognition*, and *Royal Society Open Science*.

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IDENTIFYING AND RAISING ADAPTABLE ANIMALS

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Poor welfare can stem from a mismatch between challenges in the captive environment and those the animal, having evolved in a particular natural environment, is adapted to face. Most efforts to improve animal welfare involve reforming housing and husbandry to fit the animal. A complementary solution is to choose, or mold, the animal to better fit the environment. However, traits that are beneficial in one environment may be harmful in another, so this approach runs the risk of creating “specialist” animals, fit for only one specific environment. An alternative, then, is to promote robust or “generalist” animals, adaptable enough to maintain good welfare in a wide variety of different environments. This is an important “future-proofing” measure, as production practices will continue to evolve in unpredictable ways, due to factors including shifting social expectations, technological developments, and climate change.

In past research on American mink, we examined different potential determinants of robustness. One project focused on individual differences, aiming to identify predictors of successful carnivore rehabilitation, modeled by providing environmental enrichment to barren-housed, stereotypic adult mink. Neither the degree to which stereotypies were predictable in form, nor perseveration assessed in operant tests, nor the frequency of and motivation for enrichment use, predicted which individuals showed the largest decreases in stereotypies. Formerly highly stereotypic individuals were surprisingly flexible, adapting to their new environment by reducing activity and resting in new areas, inaccessible to human handlers. Another project focused on developmental factors, aiming to test the hypothesis of “play as training for the unexpected” by examining associations between juvenile play and later responses to threats of handling or confinement. Mink who played most frequently did not then show more confident behaviour or lower physiological reactivity. However, a stronger test of whether playing helps animals become more robust to these threats will require experimentally manipulating opportunities to play.

The primary goal of our ongoing pig research program is to characterize determinants and develop methods to promote robustness. Do some personality traits predispose animals to good welfare across all environments, and can they be selected for? Does juvenile play increase robustness, and can this be promoted by modifying juvenile housing? Finally, how does the social environment impact resistance to infectious disease, and can housing treatments that improve social dynamics also ameliorate health? We hope to find ways to create animals prepared to maintain good welfare on farms of the future, whatever these may resemble.

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DRIVING ORGANIZATIONAL CHANGE IN WELFARE PRACTICES: A STAKEHOLDER FOCUS GROUP APPROACH

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Refinement of laboratory animal practices is key to maintaining and improving good animal welfare. This addresses ethical considerations for research animal use, but also is critical for ensuring reproducibility and translatability of data obtained through work with animal models. Contract research organizations can be complex businesses with multiple facilities in different countries or regions each with diverse operating requirements based on the regulatory or compliance environment, the type of research being conducted, the species worked with, and the business units and study sponsor requirements. To advance behavioral management programs for different species, our corporate welfare group has developed a process for internal consensus building for change through the use of stakeholder focus groups. Welfare working groups are species-specific, allowing participants to hone in on facilities, practices and procedures for one species at a time. Participants are recruited from different positions and business units within the company to ensure a holistic approach to refinement that is applicable across sites and regulatory and compliance environments. The work of the welfare working groups is not comprehensive, but instead focuses on areas of discrepancy in practices or areas where evidence is available to suggest changes are needed or where there have been welfare-related problems in the past. Specific topics may include behavioral management, habituation, training, low stress handling, housing, restraint, social behavior, euthanasia, welfare assessments, and routine animal care or study practices, such as bleeding techniques. Participants are selected for subcommittees based on their interest and expertise. The welfare working groups operate within their subcommittees to benchmark current practices, review scientific literature, and develop global recommendations for refining species care, management and practices. Of critical importance is the concept of consensus building within these focus groups and then amongst site operations management, veterinary teams, animal ethics committees, and senior leadership. There is also a pragmatic recognition that changes that need to be implemented at one or more sites may be asynchronous because of capital expenditure requirements, training needs or other issues. Recommendations also drive internal research and can be revisited during internal welfare assessments of sites to monitor progress in addressing the agreed upon refinements. A multinational approach to the welfare working groups encourages diverse perspectives and ideas that have a positive impact on company operations, clients, economics, and most importantly, animal welfare. An example of the Pig Welfare Working Group will be discussed.

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CHANGES IN SEPARATION RELATED BEHAVIOUR IN PUPPIES AGED FROM 12 WEEKS TO 6 MONTHS OF AGE: DESCRIPTION OF A 'GENERATION PUP' COHORT

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Understanding how canine separation related behaviours (SRBs) change over time could help with time-specific targeted behavioural interventions. We compared owner-reported data from a birth cohort study ('Generation Pup') about puppy reactions to being left alone at 12-weeks and 6-months of age. The reactions included barking, howling, whining, chewing items other than toys, and scratching at furnishings or doors. More subtle behaviours such as pacing, panting or salivating were not included because owners could not hear them after leaving, or observe their effects after returning.

Of the original 1987 dogs included in the raw data set, 284 dogs had been left alone at 12-weeks of age and had sufficiently complete data to be included in analyses. Descriptive statistics are shown in Table 1.

Separation related behaviour	12-weeks			6-months		
	Yes	No	Percentage performing the behaviour	Yes	No	Percentage performing the behaviour
Barked	22	262	7.7	62	222	21.8
Howled	10	274	3.5	4	280	1.4
Whined	72	212	25.4	28	256	9.9
Chewed	28	256	9.9	73	211	25.7
Scratched	7	277	2.5	26	258	9.2

Table 1. Number of dogs reported to perform, and not to perform each SRB at 12-weeks and at 6-months.

At 12-weeks, 35% of dogs showed SRBs, compared with 47% at 6-months. At 12-weeks, 70 dogs (25%) were reported to perform only one SRB and 30 dogs performed two or more SRBs. At 6-months, 85 dogs only showed one SRB (30%) and 48 dogs performed two or more SRBs. While more puppies showed one or more SRBs at six-months compared to 12-weeks of age, this difference was not significant.

There was a significant positive correlation between the number of SRBs dogs displayed at 12-weeks and the number of SRBs displayed at 6-months (Pearson Correlation = 0.144, $p = 0.015$), suggesting that individual dogs were usually consistent in whether they performed many or few SRBs for their age. Overall, whining significantly decreased over time, whereas barking, chewing and scratching significantly increased over time, and howling was infrequently reported and seemingly unaffected by time (Table 1). Generalized Estimating Equations showed some interactions between age, sex and breed on SRBs, which will be discussed.

Owner report is unlikely to capture the full range of behavioural signs shown by dogs when left alone. Nevertheless, the results here reveal age-related changes in some SRBs that owners can observe, potentially reflecting changes in tolerance in being left alone over time but also changes associated with development such as activity levels and teething behaviour. How the type of behaviour shown by dogs when left alone relates to welfare significance is currently unclear, but dogs appear to show consistent individual differences in the relative number of SRBs performed at both ages. In addition, further research is needed to investigate both risk factors for SRBs and interventions to improve welfare of dogs when left alone.

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OWNER-REPORTED POST-NEUTERING CARE AND WOUND PROBLEMS FOR DOGS AT AGES 7 AND 15 MONTHS

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Post-operative care following surgical neutering of dogs is understudied. This study explored post-operative care between sexes and two age groups within a cohort of neutered dogs.

Data came from an ongoing UK/ROI dog longitudinal study. Owners reported use of post-operative medication and post-operative protection (such as t-shirts or Elizabethan collars) and wound healing in surveys for dogs aged 7- and 15-months. Chi-square test analysis compared outcomes between the age groups and sexes.

A significant difference in types of post-operative protective clothing worn between sexes was identified at 7- ($X^2_{df(4)} = 28.99$, $p\text{-value} < 0.001$) and 15-months ($X^2_{df(4)} = 14.7$, $p\text{-value} = 0.005$), but not between age-groups. Post-surgery, on average 29.1% ($n=87/299$) and 48.0% ($n=129/269$) of female and male dogs respectively wore Elizabethan/buster collars, 45.5% ($n=136/299$) and 25.3% ($n=68/269$) wore t-shirts, 24.4% ($n=73/299$) and 21.6% ($n=58/269$) wore nothing and 1% ($n=3/299$) and 5.2% ($n=14/269$) wore 'other' protection.

The differences in use of post-operative medication to be given at home between age groups or sexes were not significant. At 7- and 15-months respectively, owners of 82.3% ($n=251/305$) and 83.8% ($n=207/247$) of dogs respectively were given medication to administer at home, and 17.7% ($n=54/305$) and 16.2% ($n=40$) were given no medication to administer at home.

At 7 and 15 months respectively, 75.7% ($n=253/334$) and 75.6% ($n=217/267$) wounds were reported to have healed without problem, whereas 81 and 76 had healing problems (e.g. swelling, redness, wound infection, failure of stitches). There was a significant difference between sexes in the number of wound healing problems at 7-, but not 15-months ($X^2_{df(1)} = 4.82$, $p\text{-value} = 0.03$). At 7-months 18.1% ($n=25/138$) of male and 28.6% ($n=56/196$) of female dogs had issues with wound healing.

The difference in healing between age groups approached statistical significance for male, but not female dogs ($X^2_{df(1)} = 3.7$, $p=0.054$), with 18.1% ($n=25/138$) of male dogs experiencing wound healing problems at 7- and 27.8% ($n=40/144$) at 15-months.

This study identified significant differences in post-operative care and healing between sexes. In our sample 25% of dogs were reported by their owners to experience problems with wound healing post neutering surgery, suggesting further support to owners regarding post-operative management could be beneficial.

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DISCREPANCY BETWEEN LEGAL AND ACTUAL AGE OF PUPPIES IN CROSS-BORDER TRADE

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All dogs crossing EU borders must be adequately vaccinated against rabies. Dogs younger than 15 weeks cannot travel across borders, since a primo-vaccination is only possible from 12 weeks and must be followed with a validity period of 3 weeks. However, it is said that underaged puppies are being traded with falsified passports and inadequate vaccinations, causing serious implications for their welfare and for public health. This study was undertaken to assess whether the age characteristics of imported puppies, certified to be at least 15 weeks old, matched data obtained from literature as well as with the characteristics of locally raised pups of the same breed.

Visual control was performed to evaluate the eruption status of the dentition of 10 golden retriever puppies and 10 Pomeranian puppies with alleged age between 119 and 129 days that were imported from 5 different breeders of 3 eastern European countries and that were advertised for further sale by 4 different breeder-merchants in Flanders. The dental status was also studied in carcasses of 6 Pomeranian puppies, coming from 4 eastern European countries and sold by 4 different breeder-merchants in Flanders, that had died of unrelated causes and were presented for autopsy, with a passport age between 112 and 127 days. Next, bone age of the Pomeranian carcasses was determined by studying the presence of ossification centers on craniocaudal/dorsopalmar and lateral radiographs of the elbow and carpal region. The outcome for each puppy was compared with the established reference intervals.

The passport age was found to exceed the estimated dental age interval in 6 Golden Retriever puppies and the estimated bone age interval in 5 of the Pomeranian carcasses, indicating that the certified age might have been falsified and that these puppies were actually younger than mentioned on their passports. All Pomeranians displayed a complete deciduous dentition, placing them in a wide dental age interval that lasts until first dental exfoliation and during which dental characteristics remain the same. This might have masked age fraud in those puppies where only the dental status was checked. The outcome of the carcass study demonstrates that in such cases, bone age assessment can narrow down the estimated dental age interval.

This study demonstrates that age assessment of puppies is essential in a forensic scenario and is ideally performed by combining dental age and bone age estimation. More breed specific data and data on intermediate stages are needed.

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BEHAVIOURAL DIVERSITY OF CAPUCHIN MONKEYS (*SAPAJUS LIBIDINOSUS*) IN REHABILITATION

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Behavioural diversity indexes (BDIs) have been used recently in animal welfare and behaviour studies to investigate the effects of certain animal management practices aimed at modifying behaviour and increasing welfare, such as environmental enrichment. BDI scores are expected to be high in positive welfare conditions, as there seems to be an inverse relationship between behavioural diversity and stress. Hence, BDI scores are expected to increase as positive welfare increases. Here we investigate the use of a behavioural diversity index (Shannon H index) as a way to support progress monitoring and pre-release assessment of primates rescued from the pet trade and rehabilitated for reintroduction to the wild. Sixteen bearded capuchin monkeys (*Sapajus libidinosus*) confiscated from the pet trade and housed in 25m³ enclosures in a Brazilian rescue centre were studied. The four-month rehabilitation programme consisted of providing an adequate diet and housing whilst maintaining the animals in species appropriate social groups and avoiding human interaction as much as possible. We hypothesised there would be an increase in behavioural diversity when comparing the start and the end of the rehabilitation programme. We performed 47.5 hours of observation divided into three phases (baseline, second, and final) and calculated the BDIs for the baseline (B) and final (F) phases. To test whether BDIs had increased, we calculated the difference between the final and baseline BDIs by subtracting B scores from F scores (F-B scores) and performed a one sample t-test which showed the scores calculated were significantly different from zero ($t = -3.45$, $p = 0.004$) i.e. increased significantly from the baseline (B) to the final phase (F). Moreover, a two-way ANOVA showed there were significant differences related to sex class ($F = 5.65$, $p = 0.035$), with males ($n = 10$) having significantly higher F-B scores than females ($n = 6$). No significant differences related to age nor age and sex were found. Our results suggest an association between time spent in the rehabilitation programme and an increase in the Shannon H index. Further work is needed to confirm whether this is a causal relationship, and whether it is related to independent measures of welfare. If so, the index could help with welfare monitoring of rehabilitant primates before release.

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**"IF YOU JUDGE A PIG BY THE TAIL" - HOW A SOLUTION-DRIVEN APPROACH CAN OPEN UP
NEW POSSIBILITIES TO ANIMAL BEHAVIOUR AND WELFARE RESEARCH**

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Animal welfare science covers a vast landscape of species, subjects and approaches. One approach is to be solution-driven, aiming to solve a given problem which impairs animal welfare under the status quo. In pig farming, two prominent problems can be identified. For growing pigs, it is the occurrence of tail biting, currently controlled by routine tail docking, a painful procedure; for breeding pigs, it is group housing to minimise confinement and manage aggression. Pig producers in the EU face enforcement of the ban on routine tail docking, but one significant challenge is how to prevent excessive tail biting in existing fully slatted systems without docking. I found that although different types of wood varied in their attractiveness for docked pigs, even the most favourable kind, combined with high dietary fibre, was not effective to control tail biting in undocked pigs. Instead, a combination of various edible, destructible and manipulable materials that are compatible with fully slatted systems was needed to keep tail biting at a manageable level. Although the core of this investigation was solution-driven, as the work progressed, novel ideas were also derived; I considered the longer term psychological and behavioural impact of tail biting on finishing pigs, and the association between tail lateralisation and tail biting. These investigations were developed as a result of extensive behaviour observation and contact with both docked and undocked pigs. For post-weaned sows, housing methods also require a solution-driven approach in response to new legislations to minimise confinement for breeding farm animals (e.g., Proposition 12 in California). This period is sensitive since most sows are going through considerable loss of body condition post-lactation and stress of separation from their piglets, and within days starting to experience oestrus. These physical and psychological changes can exacerbate if housing is suboptimal. Currently little is known about the psychological aspect of post-weaned sows and how they prefer to be housed in this crucial period. Long-established methods of behavioural testing, and recently developed Qualitative Behaviour Assessment, can become tools to provide insights. Both solution-driven research projects created opportunities to develop new ideas in behaviour and welfare research and allowed different ways to approach the main question. I therefore suggest that working under a solution-driven premise should not limit how we approach animal welfare science; on the contrary, inspiration and novel perspectives will always come from close working with animals and dissection of a problem to its core.

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**SYSTEMATIC REVIEW OF ANIMAL-BASED INDICATORS TO MEASURE THERMAL, SOCIAL,
AND IMMUNE-RELATED STRESS IN PIGS**

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Intensification of pig production has increased the exposition of animals to stressful conditions that might be detrimental to their welfare and their productivity. Some of the most common sources of stress in pigs are extreme thermal conditions (thermal stress), density and mixing at housing (social stress), or exposure to pathogens and other microorganisms that may challenge their immune system (immune-related stress), among others. The stress response can be monitored through indicators based on the animal coping mechanisms towards specific environmental conditions. These animal-based indicators may support decision making to keep animals' welfare and productivity. This study systematically reviewed the animal-based indicators of social, thermal, and immune-related stress in farmed pigs and the methodologies to monitor them. For this aim, the indicators of the stress models (thermal, social, and immune-related) were searched in three literature search engines, through keyword combinations according to each stress model. Manuscripts that fitted the search criteria were processed to extract data such as the animal-based indicators, the methodology, and number and kind of animals used in the study. For the thermal stress model, the region where the study was carried out was reordered, and for the immune-related stress model, the immunologic challenge used was taken into consideration. According to our results, body temperature through rectal measurements was the most implemented methodology to evaluate thermal status in pigs (87,62%) from 144 studies that studies thermal stress. From 197 studies monitoring social stress, observation of aggressive behaviors were the most frequently used indicators (81,81%). Cytokine concentration from blood samples was the most widely used indicator to monitor immune-related stress situations among the 535 publications examined (80,1%). Some animal-based indicators such as blood urea nitrogen, cytokines levels, cortisol measurements, plasmatic glucose, intestinal integrity measurements skin temperature through infrared thermography, body temperature through rectal measurement, and respiration rate through flank movements, were commonly used among the three stress models. A remarkable finding was the recent growth of alternative less-invasive methodologies to measure animal-based indicators such as cortisol in saliva, skin temperature and respiration rate through infrared thermography, and different animal welfare threats through vocalization analysis. Non-invasive methods may increase the monitoring capacity without disturbing the animals, which may refine the sampling process. The information reviewed in this study is used to discuss the feasibility and most reliable methodologies to monitor the impact of relevant stressors commonly challenging the welfare of farmed pigs.

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